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## MECHANISM OF THE FORMATION OF PURE CHOLESTEROL GALLSTONES

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AND

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Stones formed in the gallbladder without the coexistence of inflammation resulting from infection are usually designated as "pure" stones, for example, "pure cholesterol stones" or "pure calcium pigment stones." The cholesterol stones, which are by far the most common example of this type, consist largely of an enmeshing network of radial crystals of cholesterol, together with small amounts of alkali and calcium cholates and bile pigments. Since the pure cholesterol stones always occur singly, Meckel von Hemsbach<sup>1</sup> called them cholesterol solitaires.

In a recent study of certain colloidal phenomena which occur in the formation of gallstones accompanied by infection,<sup>2</sup> it was disclosed that there was no satisfactory mechanism to explain the formation of pure cholesterol stones. In the present article an attempt is made to give the experimental basis of a colloidal theory of the process of formation of such concretions.

Although the colloidal theory of the formation of gallstones is largely the product of recent investigations, it should be recalled that what is now termed colloidal behavior was recognized as important in producing concretions by Hippocrates, the Father of Medicine, in the fourth century B. C., and by Galen in the second century of our era. These renowned physicians of ancient Greece attributed the abnormal deposits to an accumulation of mucus which clung to the organ and served as a nucleus for the stone which subsequently formed. The first experimental evidence of the rôle which colloids may play in the formation of the concretions was obtained in 1684 by von Heyde, who dissolved out the crystalline material from a urinary calculus and observed a residual framework. This organic framework was recog-

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From the Department of Chemistry, The Rice Institute.

Read before the Division of Biological Chemistry at the Washington Meeting of the American Chemical Society, March 29, 1933.

1. von Hemsbach, Meckel: *Mikrogeologie*, 1856.

2. Weiser, H. B., and Gray, G. R.: *J. Phys. Chem.* **36**:286, 1932.

nized clearly by Meckel von Hemsbach,<sup>1</sup> as evidenced by the following quotation:

Two basic factors underlie the formation of every true gall or urinary stone; first, the presence of an organic substance, mucus, in which there may be deposition of salts; second, a suitable urinary or gall fluid to serve as the mother liquor for these sediments. The decomposable organic substance, mucus, is unquestionably necessary, because urinary salts and gall substances of themselves can yield only crystalline, pulverulent or granular precipitates and never larger pieces. Stones are formed only when an organic binder is carried down too.

Although the presence of a colloidal organic binding material resulting from an inflammatory process has been definitely established as essential for the formation of certain types of concretions, it was demonstrated almost a quarter of a century ago by Aschoff and Bacmeister<sup>3</sup> and by Schade<sup>4</sup> that both gall and urinary calculi may form under suitable conditions without inflammation as a result of infection.

The incidence of pure cholesterol stones is believed by Aschoff<sup>5</sup> to result from a disturbed metabolism which gives an abnormally high content of cholesterol in the blood and subsequently in the bile. According to Dostal and Andrews,<sup>6</sup> a survey of the experimental evidence does not support this view; on the contrary, it shows the absence of any connection between the cholesterol content of blood and bile. Recently, however, Wilkie and Doubilet<sup>7</sup> at McGill University made striking observations which support Aschoff's theory. Thus it was shown that in normal animals with the cystic duct tied, cholesterol passes from the blood through the mucosa of the gallbladder into the bile provided the cholesterol concentration of the bile is lower than that of the blood, whereas cholesterol passes from the bile through the gallbladder into the blood stream provided the cholesterol concentration of the bile is higher than that of the blood. Moreover, the amount as well as the direction of passage of the cholesterol appears to depend on the blood-bile cholesterol ratio.

In contrast to Aschoff's theory, Naunyn<sup>8</sup> attributed the presence of excess cholesterol which may lead to the formation of stones in the gallbladder to the disintegration of the epithelium of the gallbladder or to the direct secretion of cholesterol by the mucosa of the gallbladder.

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3. Aschoff, L., and Bacmeister, A.: *Die Cholelithiasis*, Jena, G. Fischer, 1910. Kleinschmidt, O.: *Die Harnsteine*, Berlin, Julius Springer, 1911.

4. Schade, H.: (a) *Kolloid-Ztschr.* **4**:175, and 261, 1909. (b) Alexander, J.: *Colloid Chemistry* New York, The Chemical Catalog Company, 1928, vol. 2, p. 803.

5. Aschoff, L.: *Lectures on Pathology*, New York, Paul B. Hoeber, Inc., 1924, p. 206.

6. Dostal, L. E., and Andrews, Edmund: *Arch. Surg.* **26**:258, 1933.

7. Wilkie, A. L., and Doubilet, Henry: *Arch. Surg.* **26**:110, 1933.

8. Naunyn, B.: *Klinik der Cholelithiasis*, Leipzig, F. C. W. Vogel, 1892.

This view has been largely disproved, and has been abandoned by most pathologists. Recently, however, it has been revived by Elman and Graham<sup>9</sup> to account for the presence of cholesterol crystals in the walls of the gallbladder. Although this pathologic condition called cholesterosis or "strawberry" gallbladder is well known, it does not follow that the crystals deposited in the mucosa of the gallbladder are secreted by the mucosa.

On one point everybody is agreed: Whatever the source of the excess cholesterol, a condition must arise which causes precipitation of the compound or no stone can form. During stasis, Aschoff visualizes the precipitation from a hypercholesterolated bile about some nucleus, ultimately leading to the formation of a gallstone. While this step is necessary, pathologists have not always recognized that precipitation is in itself altogether inadequate to account for the binding of the minute crystalline particles into a concretion. Schade<sup>10</sup> realized the necessity of accounting for the collection of the precipitated cholesterol into a coherent mass and proposed the following mechanism:

Bile always contains, in addition to cholesterol, small quantities of fat dissolved in its cholate. Now in stasis of the bile, as the experience of the surgeon and the pathologist proves, the concentration of the cholate is gradually diminished by autolysis and resorption until finally a water-clear, almost cholate-free fluid is left; but the cholesterol remains in undiminished quantity and is ultimately in excess. The increasing impoverishment of the bile in cholate content, compels small quantities of cholesterol to separate out from time to time. But owing to the presence of fat it is guttulate separation which occurs, and since in such simple stasis, foreign substances are lacking, there is nothing to prevent the aggregation of the droplets.

While Schade's proposed mechanism possesses elements of value, it is based on certain misconceptions which render it inadequate. In the first place, it is taken for granted that the alkali cholates can hold large amounts of cholesterol in the dispersed state; but it requires about 40 parts of cholate to disperse 1 part of cholesterol. In the next place, it is assumed that during stasis the cholates gradually disappear, allowing the cholesterol to precipitate. But Rous and McMaster<sup>10</sup> and more recently, Andrews, Dostal, Goff and Hrdina<sup>11</sup> demonstrated conclusively that stasis alone does not necessarily alter the bile salt-cholesterol ratio in the gallbladder. The introduction of some other factor such as bacterial infection or chemical irritation, say by pancreatic juice, is necessary for resorption of the bile salts to take place. But inflammation from infection, or, indeed, anything more than a relatively mild chemical inflammation, is not essential for the formation of pure cho-

9. Elman, Robert, and Graham, E. A.: *Arch. Surg.* **24**:14, 1932.

10. Rous, Peyton, and McMaster, P. D.: *J. Exper. Med.* **34**:47, 1921.

11. Andrews, Edmund; Dostal, L. E.; Goff, M., and Hrdina, L.: *Ann. Surg.* **96**:615, 1932.



lesterol solitaires, since gallbladders in which they are found usually show no signs of inflammation and the pathologic results of infection are absent. Moreover, in the presence of infection, stones of a distinctly different type are formed. Finally, emphasis is laid on the importance of the presence of excess fat; but cholesterol is quite soluble in fat, and an excess of the latter would tend to prevent rather than favor the formation of gallstones.

#### EXPERIMENTAL WORK

The following experiments appear to throw considerable light on the mechanism by which precipitated cholesterol may be collected into a unified coherent mass:

1. It is commonly stated that cholesterol, which is quite insoluble in water, is held in the dispersed state in the bile by the peptizing action of the alkali cholates. This statement we have found to be only partly correct. Fat-free cholesterol was digested for several days in a 6 per cent solution of sodium glycocholate kept at 37 C., the body temperature. The amount taken up corresponds to but 0.2 Gm. in 100 cc. of solution or 1 part of cholesterol to 30 parts of bile salt. In contrast to the relatively low peptizing action of the cholates is the marked solubility of cholesterol in fats. Thus, at body temperature, 100 cc. of olive oil dissolves 6 Gm. of cholesterol. Since normal bile may contain 1 per cent or more of fatty material, it is apparent that from one-third to one-half the cholesterol in normal bile from the common duct<sup>12</sup> is dissolved in the emulsified fat. Moreover, any excess cholesterol tends to concentrate around the fat droplets.

It is significant that the cholesterol which separates from a supersaturated solution in fat comes out in the form of needle-like interlacing crystals and not in the form of thin platelets such as those obtained from the alcoholic solution.

2. A drop of olive oil was shaken with 10 cc. of a 6 per cent solution of sodium glycocholate. A stable emulsion was formed in which the droplets varied in size from extremely minute to fairly coarse. Figure 1 *A* shows the appearance of a portion of this emulsion magnified 70 times. The emulsion is quite stable but tends to "cream" on standing, giving an upper layer that is relatively richer in fat than the lower layer.

3. A drop or two of olive oil saturated with cholesterol at 50 C. was added to 10 cc. of the solution of sodium glycocholate at 25 C. and the mixture shaken vigorously. An emulsion was again formed, but this time there was present an amount of free cholesterol in excess of the amount required to saturate the fat at 25 C. A small portion of this excess cholesterol was peptized by the alkali cholate and the remainder concentrated on the surface of the droplets of emulsified fat. This is shown clearly in figure 1 *B*. It is interesting to note that under these conditions the fat droplets have an "armor plate" of cholesterol, as evidenced by the fuzzy appearance of the edges of most of the fat droplets and by the distortion in shape of many of them. It is apparent that cholesterol has a marked tendency to collect at an oil-water interface. For this reason, in the bile excess cholesterol over and above that dissolved in the bile fat and peptized by the alkali cholates concentrates at the surface of the droplets. It thus appears that the chief rôle of the bile cholates in holding relatively large amounts of cholesterol in colloidal

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12. The cholesterol content of normal bile from the common duct is between 0.06 and 0.12 per cent.

dispersion is as an emulsifying agent for the fats, thereby furnishing a relatively large surface on which minute particles of cholesterol are absorbed and prevented from settling out.

4. Like most emulsions, those considered in the preceding paragraph "cream" on standing; that is, the upper portion becomes relatively richer in fat. This process is accompanied by some coalescence, especially of the larger drops, into still larger units. When the droplets are coated with a film of cholesterol there is a marked tendency to form clumps of droplets. Thus, after an emulsion containing excess cholesterol has stood for a day or two, it is apparent that a considerable portion of the cholesterol has collected in an upper cloudy layer. Examination under the microscope reveals the presence of clumps of droplets

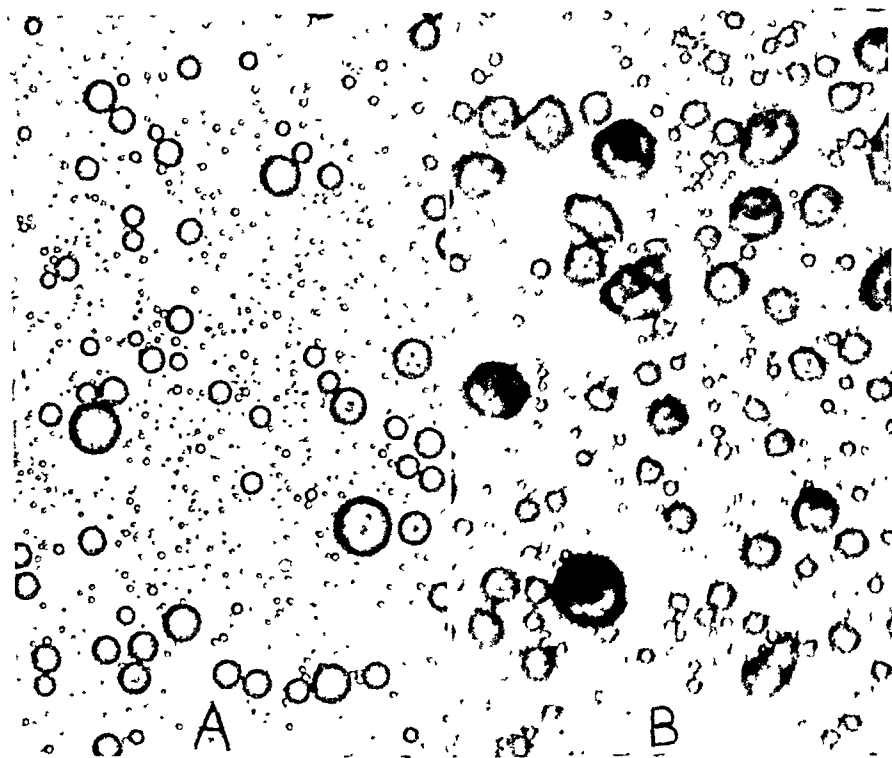


Fig. 1.—Emulsions of olive oil with sodium glycocholate: *A*, in the absence of cholesterol; *B*, in the presence of excess cholesterol;  $\times 70$ .

coated with cholesterol so finely divided that no crystal structure is visible. A typical clump of this kind is shown in figure 2 *A*. Note the irregular shape of the clump and the white color which is due to the minute particles of cholesterol in the oil-water interface.

5. For the formation of large cholesterol crystals from the minute particles, it is necessary for the emulsifying film of sodium glycocholate to be broken so that cholesterol can come in contact with the saturated fat droplets. Under such circumstances it would be expected that the minute particles would dissolve and reprecipitate out on larger units. There proved to be two relatively simple methods of breaking the glycocholate film on the droplets: First, add a trace of acid which converts the glycocholates to glycocholic acid which is not an emulsifying agent; or second, allow the sample to dry, thus causing the film of the hydrophilic emulsifying agent to crack.

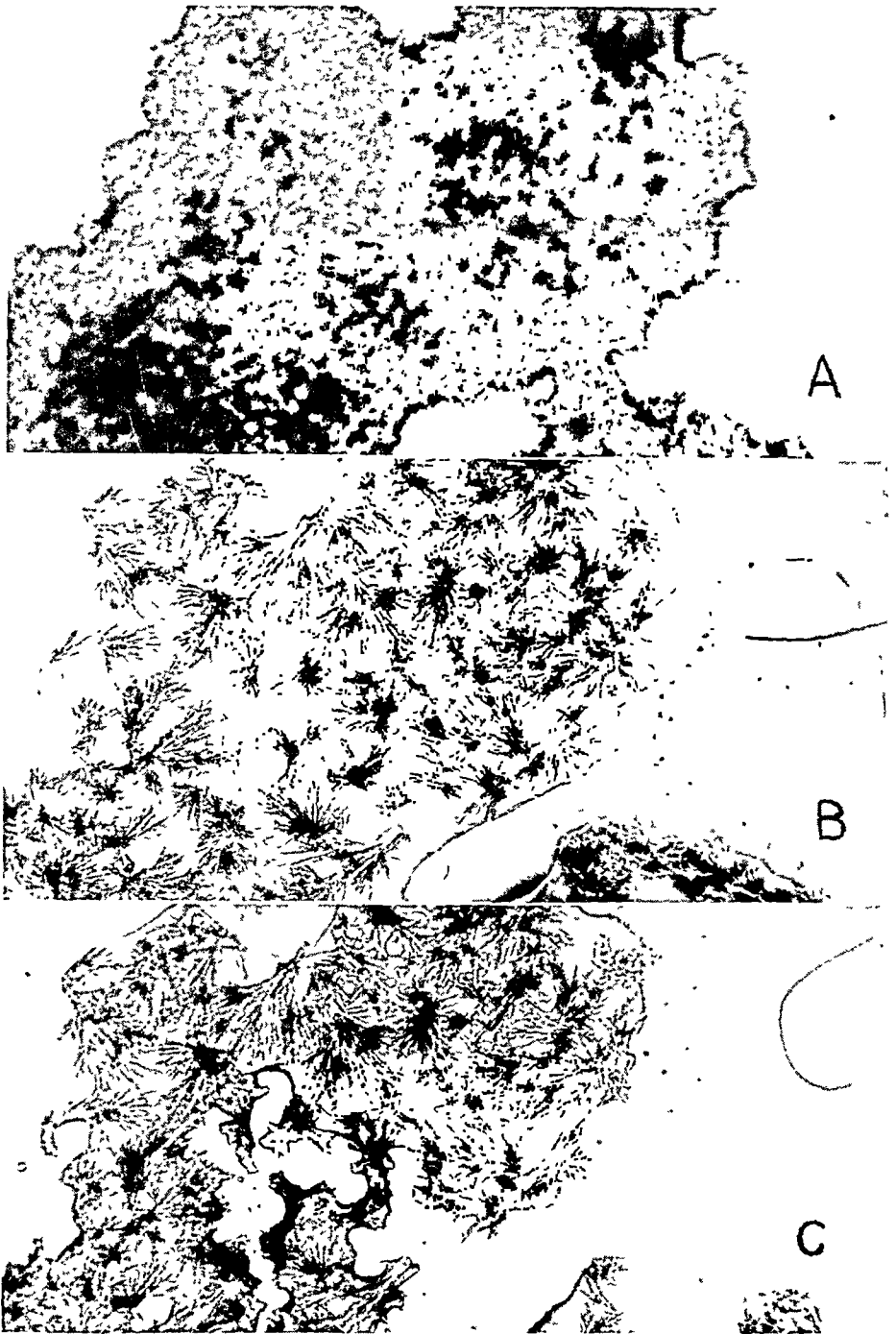


Fig. 2.—Cholesterol-fat clump: *A*, freshly formed; *B*, after four hours; *C*, after twenty hours;  $\times 70$ .

The effect of allowing the specimen shown in figure 2 *A* to stand in the air is shown strikingly in figure 2 *B* (after four hours) and 2 *C* (after twenty hours). Note the disappearance of the minute particles of cholesterol and the formation in their stead of the fanlike, feathery crystals similar in all essential respects to those which characteristically separate from the solution in fat. This process is accompanied by the release of the greater portion of the fat solvent. Note the relatively large size of the crystals, especially in *C*, and that they are suspended in a drop of fat the outline of which is clearly visible.

6. It would appear obvious that the formation of a mass of interlocking crystals by the mechanism described in the previous paragraph would yield a relatively firm "stone." This was demonstrated by pouring fat supersaturated with cholesterol into glycocholate solution, emulsifying, allowing to stand for some time, and then separating the mass of fat and cholesterol from the remainder of the emulsion by centrifugating. After absorbing the excess fat with blotting paper, it was formed into a ball and dried. The result was a relatively hard crystalline mass, simulating the natural pure cholesterol stone in appearance, composition and properties. To prepare a photograph, a small ball of the cholesterol mass was flattened out on a microscope slide, dried, and digested at body temperature for four weeks. The result is shown in figure 3. Although the magnification is only 10 diameters, the radial crystalline structure characteristic of the natural stones is clearly revealed. Figure 4 is a section of the preparation shown in figure 3, magnified 70 times. The higher magnification shows in a striking way how a coherent body is obtained as a result of the laying down of an interlacing mass of needle-like crystals.

In the light of the foregoing series of experiments, the mechanism of the formation of cholesterol stones in the absence of an inflammation due to infection is believed to be as follows:

In biliary stasis resulting from anatomic or physiologic abnormalities,<sup>13</sup> the bile collects and concentrates in the gallbladder where it may remain for a long period. During this period of stasis there may be an infiltration of cholesterol from hypercholesterolated blood and a decrease in the amount of the alkali cholates which are responsible for retaining the fat in the form of an emulsion as well as the cholesterol in the dispersed state. In the absence of infection, a decrease in the alkali cholates may result from either or both of the following causes: (1) a change in the  $pH$  of the bile from the alkaline to the acid side thereby converting the alkali salt to the insoluble glycocholic acid which is neither an emulsifying agent for fat nor a peptizing agent for cholesterol; or (2) a physiologic change in the wall of the gallbladder which allows resorption of the alkali cholates.

There is no doubt that the first factor mentioned will contribute to the precipitation of cholesterol, since bile from the gallbladder is normally acid while that from the hepatic duct is alkaline. This normal change in  $pH$  value is probably accentuated in biliary stasis.

In the normal gallbladder there is little or no resorption of alkali cholates. Since pure cholesterol stones are found in gallbladders that show no signs of inflammation, past or present, it follows that any resorption of cholates that leads to the formation of a pure cholesterol stone must result from a physiologic derangement that does not produce histologic changes. Clinical irritation that is not sufficiently severe to leave a permanent change in the tissue may be a contributing factor. Thus a reflux of pancreatic juice into the gallbladder causes

13. Aschoff,<sup>5</sup> p. 194.

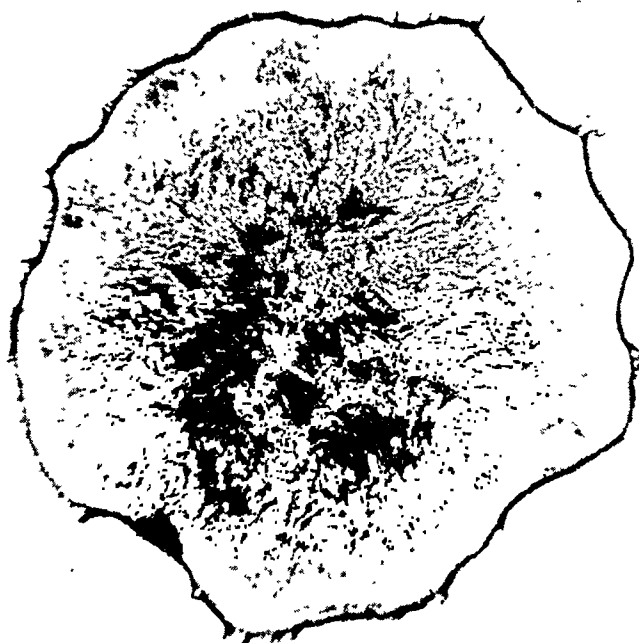


Fig. 3.—Synthetic pure cholesterol stone;  $\times 10$ .



Fig. 4.—Interlacing crystalline structure of synthetic cholesterol stone;  $\times 70$ .

cholecystitis and results in marked absorption of cholates.<sup>14</sup> The injection of bacteria produces a similar effect, but this changes the whole picture, causing lesions and leading to a different type of stone.

The disappearance of alkali cholate, either by conversion to glycocholic acid or by resorption, causes precipitation of cholesterol. This is, of course, most marked in highly hypercholesterolated bile. The excess cholesterol collects around the fat droplets which tend to coalesce as the cholate is gradually removed. Clumps of fat interspersed with cholesterol result, and the process of solution of the finely divided particles and subsequent reprecipitation in large needle-like crystals binds the mass together. The continuation of this process for a long period leads eventually to the concrement which consists of relatively large crystals of cholesterol together with a small amount of enclosed fat.

Particular attention should be called to the importance of fat in the synthesis of pure cholesterol stones. Not only does it serve as a collecting agent which brings together the particles of precipitated cholesterol but its solvent action is responsible for the growth of the interlacing crystals which bind the mass into a concrement.

#### SUMMARY

The results of this investigation may be summarized as follows:

1. Precipitation of cholesterol in the gallbladder is in itself altogether inadequate to account for the formation of pure cholesterol concretions.

2. Experimental observations have been made which furnish the basis of a mechanism to account for the formation of such concretions during biliary stasis resulting from anatomic or physiologic abnormalities.

3. By the proposed mechanism, gallstones have been synthesized which simulate the natural concretions in both macroscopic and microscopic appearance and in properties.

4. Particular attention has been called to the importance of fat in the formation of pure cholesterol concretions, both as a collecting agent for the minute particles of precipitated cholesterol and as a solvent which is responsible for the growth of interlacing crystals into a concrement.

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14. Wolfer, J. A.: Surg., Gynec. & Obst. **53**:443, 1931. Andrews, Dostal, Goff and Hrdina.<sup>12</sup>

# HISTOLOGIC OBSERVATIONS IN A CASE OF OLD GUNSHOT WOUND OF THE BRAIN

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AND

T. S. KIMBALL, M.D.

LOS ANGELES

The opportunity to study the pathologic aspects of an ancient traumatic lesion of the brain is not frequently afforded one, even though an abundance of neuropathologic material is available for study. It is perhaps for this reason that so little is known about the ultimate appearance and structure of this type of lesion. Aside from a revelation of the behavior of the individual elements after so long an interval, a more comprehensive conception of the life history of cerebral wounds is to be gained from a critical histologic study of such cases when they do come to hand.

The case here considered is of interest from the standpoint of its twenty-two years' duration, the minimal damage to the skull and dura and the comparatively uncomplicated character of the lesion produced by a bullet of small caliber. It was anticipated that an entirely quiescent lesion would be found histologically after this long interval. The indications of long-continued cellular activity and observations which suggested the fate of the various elements made it seem worth while to report the case.

## REPORT OF CASE

A Mexican laborer, aged 57, died of lobar pneumonia six days after admission to the Los Angeles County General Hospital. Twenty-two years before he had been accidentally shot in the head while hunting and had remained unconscious for three weeks thereafter. Examination revealed a depression in the skull in the upper left parietal region close to the midline. There were characteristic manifestations of a lesion of the left upper motor neuron—spasticity of the extremities on the right side, atrophy from disuse, hyperactive deep reflexes in these members and pathologic responses of the toes.

Autopsy was performed seven hours post mortem by one of us (T. S. K.). The original defect in the skull had been closed by the formation of new bone, apparently derived from the inner table. There still remained a depression in the outer table. The dura was adherent to the bone at this point. There were no adhesions between the dura and the brain. In the upper part of the left precentral gyrus, at the point of entrance of the bullet, there was found an angular hollowed defect in the cortex

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From the Departments of Neurology and Pathology, College of Medical Evangelists.

measuring 2.2 by 1.8 cm. in surface extent and about 0.5 cm. in depth. The pia-arachnoid appeared to be continuous with a thin yellowish membrane which lined the defect. A somewhat distorted 22 caliber bullet was found lodged in a conical cavity in the third right temporal convolution just in front of the preoccipital notch. This defect measured 0.7 by 1 cm. in its surface diameters.

The brain was sectioned along the course of the bullet to expose as much as possible of its track (fig. 1). The bullet, having entered the upper portion of the left precentral gyrus, had emerged from the cortex of the posterior portion of the left cingulate gyrus, penetrated the corpus callosum at the point of union of this structure with the crurae of the fornix, passed through the right lateral ventricle at the juncture of the body, posterior and inferior horns, and penetrated the substance of the right temporal lobe at this surface, where it was found on external examination.

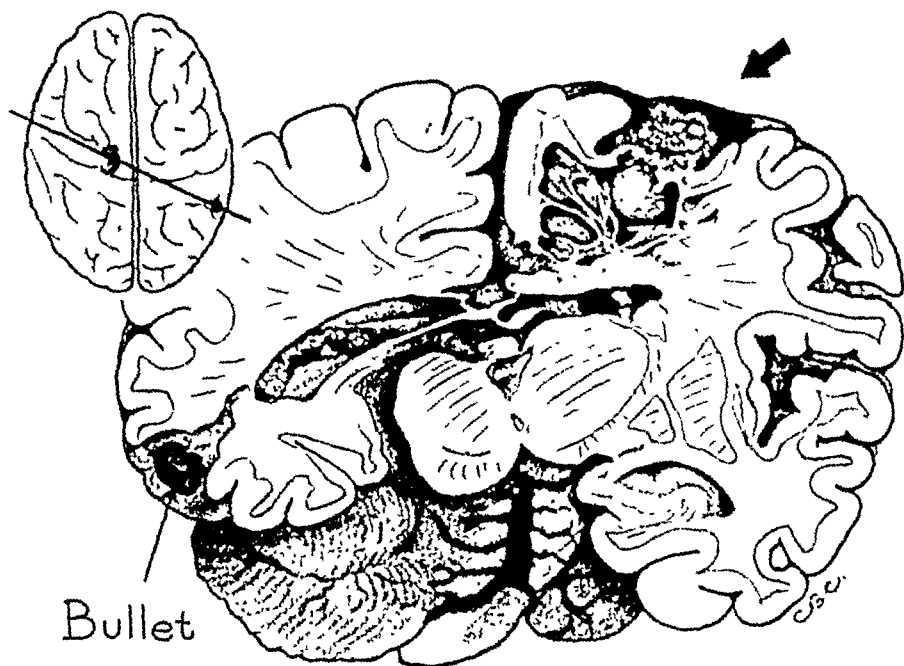


Fig. 1.—Section of the brain along the course of the bullet. The depression in the cortex at the missile's point of entrance, the subjacent multilocular cyst, the depression in the atrophied corpus callosum, the adherent choroid plexus and the cavity of final lodgment of the bullet are indicated. The insert shows the line of section.

At the point of entrance of the bullet, surrounding the depression were found multiple small cystic spaces occupying almost the entire centrum above the corpus callosum. These small spaces had evidently been filled with fluid in the recent state. The intervening walls and lining of the spaces were golden yellow. This extensive lesion was probably the result of indriven fragments and hemorrhage, in addition to the wound made by the bullet. The corpus callosum was atrophied, and the point of entrance of the bullet was marked in this situation by a depression in its upper surface. The choroid plexus was still adherent at the point of exit of the bullet from the ventricle. The cavity in the left temporal lobe in which the bullet was found was conical, with its apex at the ventricle wall and the base



at the surface of the brain. The base of the left cerebral peduncle, the left side of the pons and the pyramid were smaller than the right, owing to secondary degeneration of the motor fibers.

#### HISTOLOGIC OBSERVATIONS

Blocks of tissues were taken from the cystic area below the wound of entrance, from the cortex along the dorsomesial margin of the hemisphere above the defect, from the corpus callosum at the point of its penetration by the bullet, from the point of adhesion of the choroid plexus to the ventricular wall and from the walls of the cavity in which the missile had finally lodged. Tissue sections from these blocks were stained or impregnated with hematoxylin and eosin, scarlet red, phosphotungstic acid hematoxylin, the cyanine method for tigroid substance, the Courville-Krajian method for myelin sheaths, Krajian's silver impregnation method for reticulin, Cajal's gold chloride method for neuroglia, the reduced silver method for axis cylinders and Penfield's combined method for oligodendroglia and microglia.

*Alterations in the Nerve Cells.*—In all of the sections studied, the nerve cells, regardless of position, showed a general loss of tigroid substance. In view of the fact that the patient died of lobar pneumonia, this is only of incidental significance. The reduced silver method showed an intact fibrillar apparatus in the nerve cells at a distance, but in those adjacent to the injury, this structure was found to be undergoing regressive change or was absent altogether. In a portion of cortex cut off by the track of the bullet, the nerve cells were morphologically intact, but their fibrillar apparatus was found to be granular. In a limited portion of cortex which formed the dorsal border of the hemisphere, separated from the centrum by the multilocular cavity, the nerve cells were entirely absent.

In a small residual fragment of cortex subjacent to the wound of entrance and separated from the underlying white substance by extensive destructive change, the normal structures were entirely replaced by a loose vascular connective tissue reticulum. In the superficial portion of the opposed cortex, there was a zone of vascular connective tissue which replaced all other elements. The nerve cells in the deeper layers appeared distorted and sclerotic.

In the cortex adjoining the cavity containing the bullet, cyanine preparations revealed the nerve cells to be only faintly outlined, tigroid granules being entirely absent from their cytoplasm (fig. 2A): Some of the cells contained localized collections of pigment. The reduced silver preparations revealed a variable degree of granular disintegration of the neurofibrillar apparatus. Occasional pyknotic or "sclerosed" cells with their characteristic corkscrew apical processes were observed near the margin of the defect. Rarely a deeply impregnated, distorted form was found, the scarred remains of a ganglion cell (fig. 2B).

*Alterations in the Nerve Fibers.*—The nerve fibers separated from their nerve cells had long since disappeared, so that it was impossible to trace the course of degenerated bundles such as the motor pathway. The white substance somewhat removed from the immediate zone of injury appeared homogeneous, and individual fibers revealed no evidence of injury. In the myelin sheath preparations, as the margin of the bullet track was approached, the nerve fibers became fewer and more altered. Individual fibers were found to be altered by the formation of globular or fusiform swellings of various sizes, characteristic of fibers undergoing regressive change (fig. 2C). At times these swellings occupied the termination of the demonstrable sheath. Rarely an additional unstained portion could be discerned under reduced light, extending toward the margin of the defect. The

terminal portion was frequently found to be paler than usual, at times fragmented or segmented, and often of smaller caliber than the rest of the fiber. This terminal segment was frequently broken off sharply, with a resulting squared end, or presented an irregular, pale, expanded portion varying in size (fig. 3).

The changes in the axis cylinders were equally interesting. In the zone of degeneration these structures were found to be undergoing granular, fragmentary or "burnt string" degeneration. In expanded regions, which corresponded with the myelin swellings, were found local dispersions of the argentophilic material in the form of rings, irregular aggregations or complex masses. The impression given by these collections was that in the process of regressive change the myelin swellings had distorted the arrangement of the rows of argentophilic granules

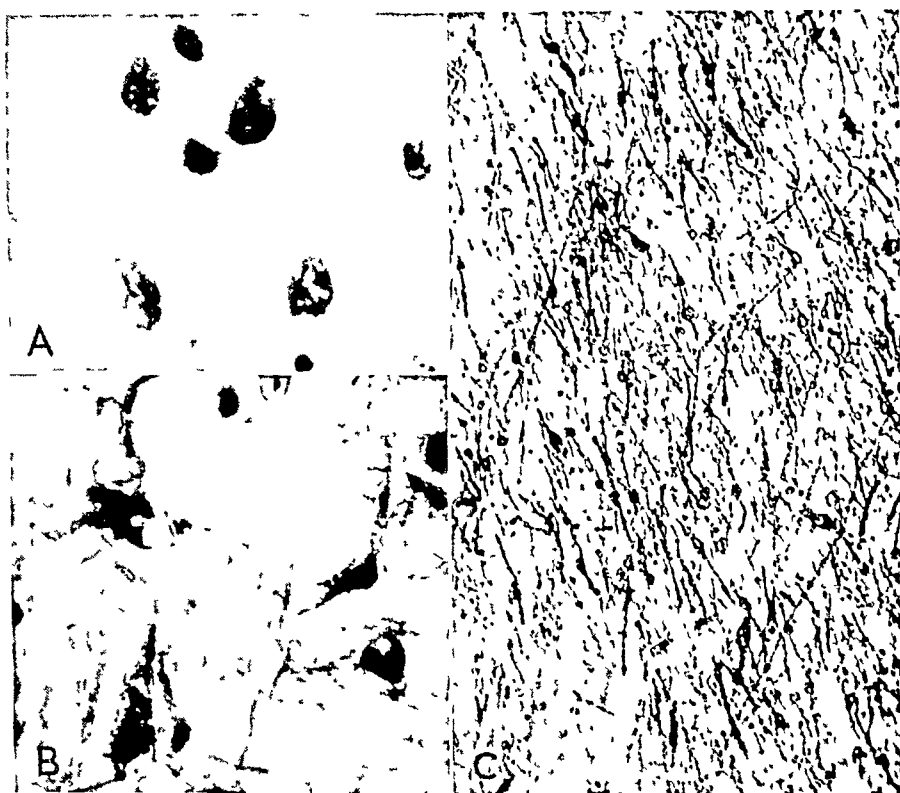


Fig. 2—Changes in the nerve elements. *A* shows ghost cells at the margin of the cavity in which the bullet was lodged. Cyanine stain for tigroid substance;  $\times 360$ . *B* shows degenerated nerve cells from the same location. A sclerosed and vacuolated cell is shown in the upper left corner. Cajal's reduced silver method;  $\times 360$ . *C* illustrates degenerating myelin sheaths from the same situation. Courville-Krajan method;  $\times 120$ .

representing the remains of the axis cylinder. When occurring at the end of a degenerating fiber, these structures gave rise to appearances of "pseudo end-bulbs."

End-bulbs which are known to persist for months or even years after the injury of the brain were not observed except in a very modified form. At the termination of the fine axis-cylinders was found a small irregular expanded portion, a spear head, a fine hairlike filament or a clump of granules. At times a row of rounded fragments gave the fiber a barred or beaded appearance. The accom-

panying drawing illustrates a number of these terminations of axis-cylinders (fig. 4). In a portion of cortex isolated from the white centrum by local cysts, the axis-cylinders of the morphologically preserved nerve cells were found to terminate a short distance below the cell in a sharp point, a late "corrosion point."

*Progress of Phagocytosis.*—Substantiating these indications of a long-continued degeneration in the nerve fibers, evidences of active phagocytosis of the products of regressive changes were to be seen. Specific stains revealed globules of free fat in wandering macrophages in the zone of degeneration, especially in the perivascular spaces. The endothelial cells of the blood vessels likewise contained fat.

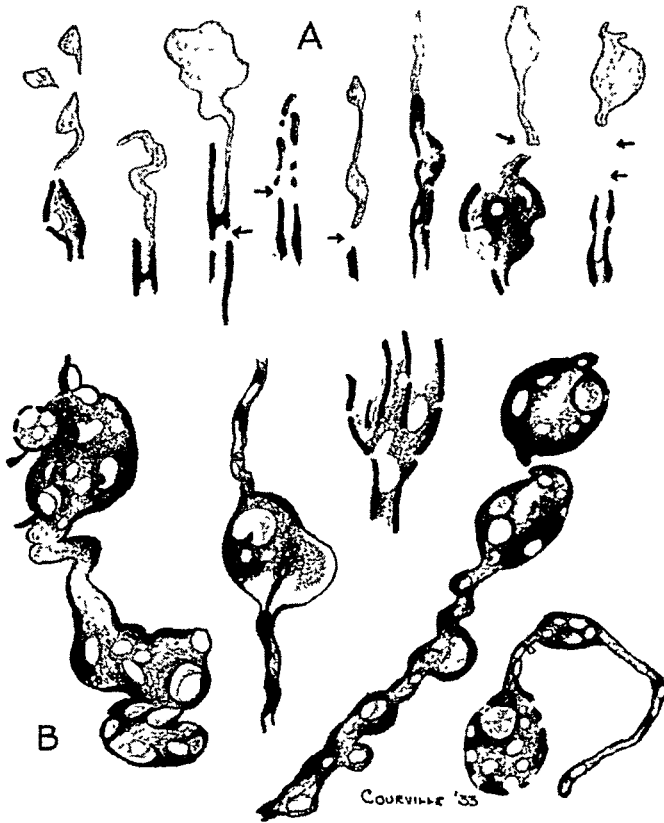


Fig. 3.—Alterations in the myelin sheaths. *A* shows the modes of termination of the fibers extending into the margin of the defect. The arrows indicate fractures in the sheath, possibly artefacts. *B* shows the detail of the globular swellings of the myelin sheaths. Drawn from a Courville-Krajian myelin sheath preparation.

The reduced silver method revealed granules of argentophilic material in free macrophages and, often in larger amounts, in the vascular endothelium.

Of particular interest in this connection was the reaction of the microglia in the cortex adjacent to the track of the bullet. These cells were found to be undergoing morphologic alteration recognized to be transitional in the formation of compound granular corpuscles. Most of the cells were in the first stage, but some had reached the "spider cell stage," and rarely an ameboid cell<sup>1</sup> was found in the reticular portion of the degenerated area.

1. Rand, C. W., and Courville, C. B.: Arch. Neurol. & Psychiat. **27**:605, 1932.

Of unknown significance was the occurrence in a reticular area of phagocytes laden with golden yellow pigment. These macrophages were found singly or in numbers, most abundant in the region of the blood vessels.

*Evidences of Attempts at Repair.*—The repair of wounds of the brain is the result of a variable reaction on the part of the astrovascular system, with additional connective tissue proliferation from the meninges, particularly the dura. The wide variation in the types of reaction observed depends on the character of the lesion.

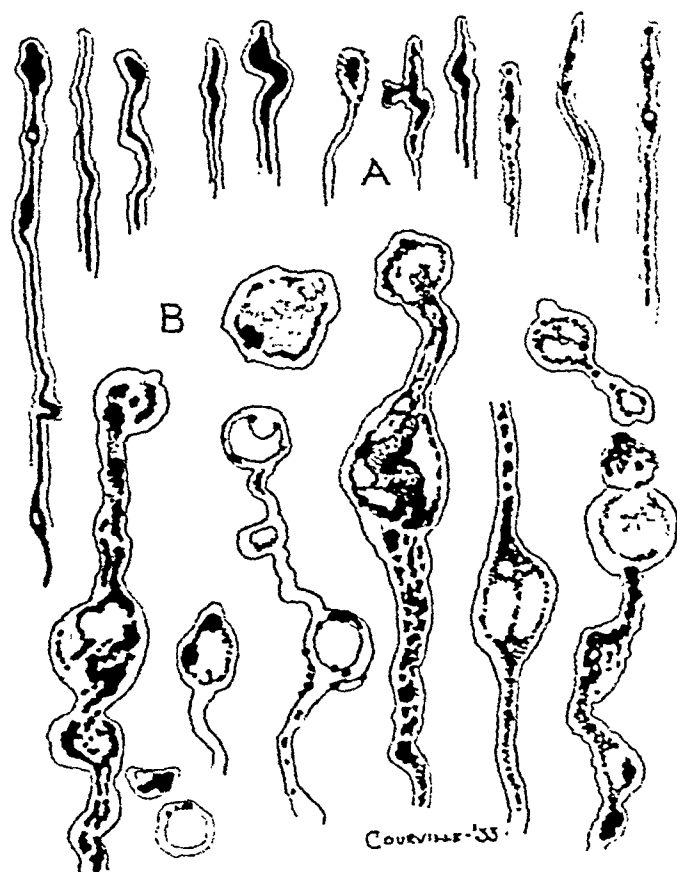


Fig. 4.—Alterations in the axis cylinders. *A* shows the types of terminations of the fibers extending into the marginal zone about the defect. *B* shows degenerating axis cylinders, illustrating the effect on the granule arrangement of the myelin swellings. Free rings and balls are also shown. Drawn from a Cajal reduced silver preparation.

*Connective Tissue.*—This tissue was found to be fairly abundant in the lower part of the wound of entrance and practically entirely absent elsewhere. As there was no adhesion between the brain and the dura, the connective tissue must of necessity have some other origin. It is likely that at the time of injury fragments of bone and dura were carried into the brain, resulting in serious local laceration. The degenerating brain tissue, and the presence of foreign bodies, together with extensive pial injury, evidently favored the development of a modified connective

tissue scar. The upper portion of the wound of entrance, composed of a multi-locular cyst, was perhaps the site of an original local hemorrhage, which did not favor the deposit of connective tissue at its margins. The tissue about the cavity in the right temporal lobe in which the bullet was lodged gave no evidence of the formation of connective tissue.

One rather unusual feature of the connective tissue reaction was its replacement of the parenchymatous elements in the residual small fragments of cortex overlying injured white substance. When extensive scar formation had replaced the injured white matter, this replacement had been quite complete. Otherwise, this vascular reticulum was rather superficial and apparently continuous with the pia mater (fig. 5 *A* and *B*).

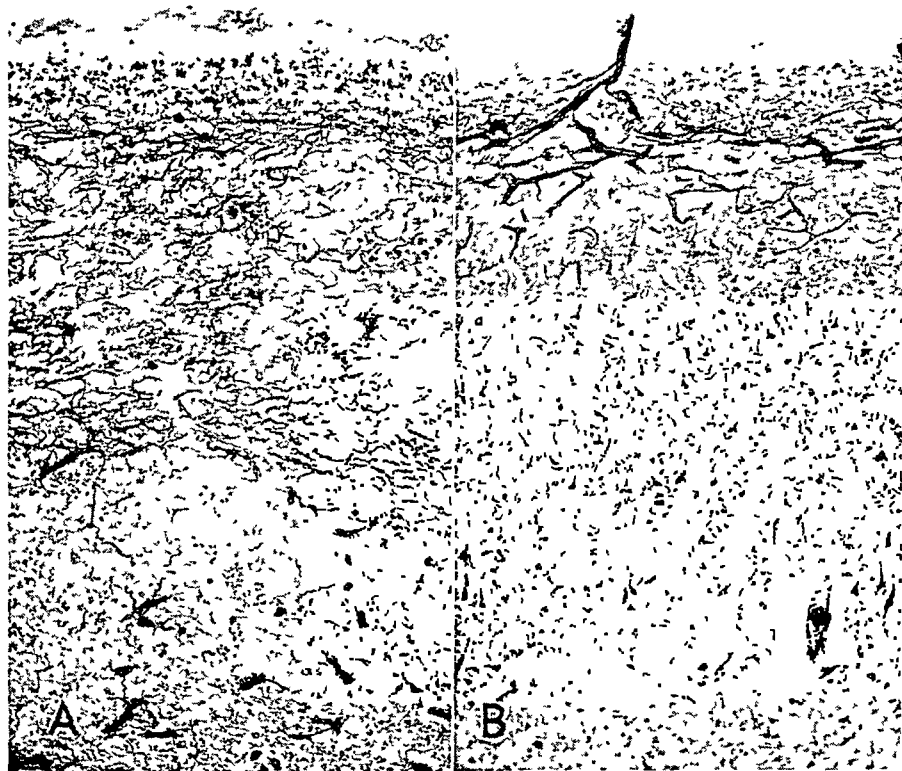


Fig. 5—Vascular scar in the cortex. *A* shows complete replacement of the cortex by a network of blood vessels over an extensively injured white substance. *B* shows a narrow superficial vascular scar on the cortex on the opposite side of the sulcus from *A*. The underlying white substance is less seriously injured. Krajian's method for reticulum;  $\times 100$ .

There was but one situation in which a combined fibro-astrial cicatrix had been formed, in the lower margin of the cavitation found beneath the wound of entrance. Here was found a fairly dense scar composed of fibers arranged in parallel bundles and running at right angles to the free margin of the wound. Extending into the adjacent brain tissue was a network of blood vessels. In the deeper portions of the scar the bundles of connective tissue fibers became intermingled with those of neuroglial origin (fig. 6).

*Neuroglia.*—The reaction of the neuroglia in the various situations along the track of the bullet was also of interest. In the denser scar, as has already been

intimated, the neuroglia fibers were intermingled with the connective tissue fibers to form closely packed fasciculi. The cell bodies of the astrocytes were elongated and lay in the bundles of hairlike fibrils. These fibers were evidently complementary to the connective tissue fibers, for they became less abundant in the superficial connective tissue portion of the scar.

In the areas which had a more reticular appearance, surrounding the more dense central cicatrix, a more typical glial scar was found. The neuroglia formed a fairly uniformly meshed network, the fibrils forming the intervening strands while the nuclei were lodged in the interstices of the mesh.

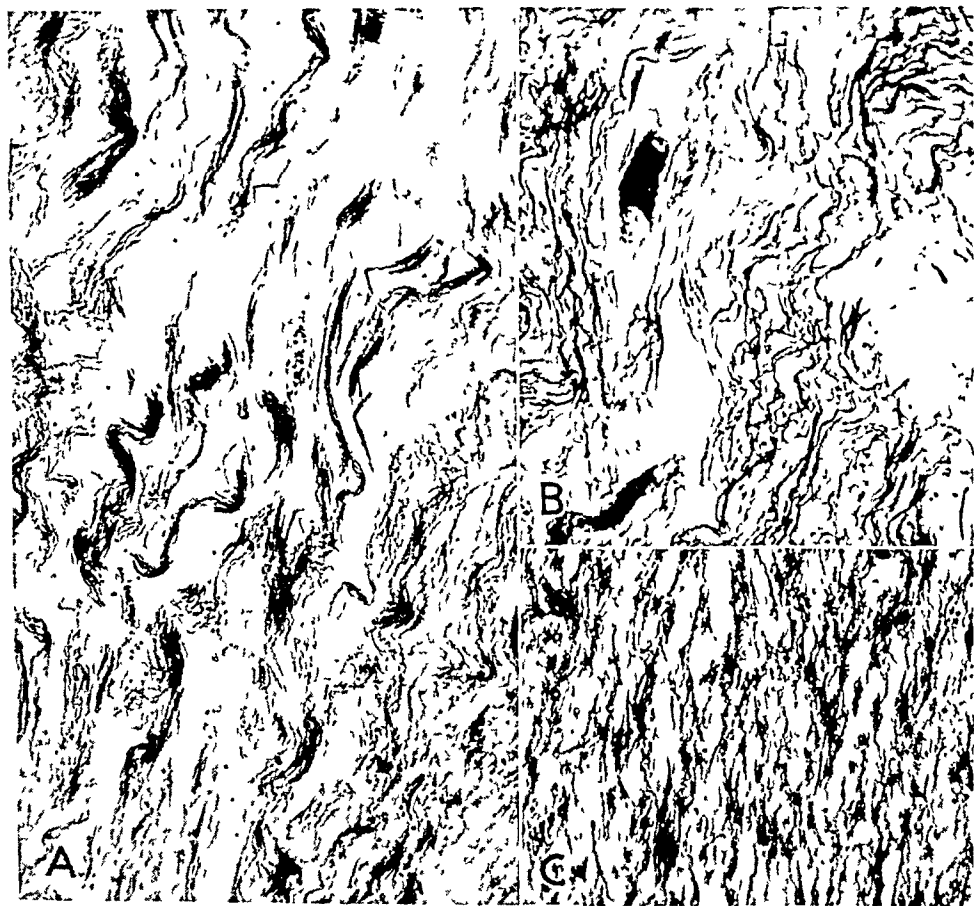


Fig 6—Composition of fibro-astral scar. *A* shows neuroglial and fibroglial fibers arranged in bundles. Phosphotungstic acid hematoxylin;  $\times 160$  *B* shows parallel strands of reticulin in the superficial portion of the wound. Krajian's method for reticulin;  $\times 160$  *C* shows piloid astrocytes of Penfield, with parallel fibrils in the deeper portion of the scar. Reduced silver method;  $\times 160$

In still other areas where the margin of the wound was more sharply defined and the adjacent tissue appeared solid well up to its borders, there was little or no neuroglial reaction. This was especially true along the upper margins of the cystlike spaces at the wound of entrance and about the cavity in which the bullet finally lodged. This variation was probably due to the amount of tissue destruction, the presence of decadent tissue encouraging the proliferation of neuroglia.

## COMMENT

It is natural to assume at the outset that a wound of the brain would ultimately become quiescent, as is the case with wounds of other tissues. This study seems to indicate that this is not the case, for after an interval of twenty-two years, the processes of disintegration, of phagocytosis and probably also of repair were still taking place. Seriously damaged nerve cells had maintained their morphologic identity throughout this long interval. The peculiarities of cicatrix formation and the significance of disintegrative changes in the nerve fibers and of pigment deposit merit consideration.

The histologic changes about the wound of entrance seem to confirm Penfield's postulates as to the mode of formation and the constituents of a cerebral cicatrix. Where there has been extensive destruction of tissue in close proximity to the pia mater and especially where the dura has been injured, a marked connective tissue and neuroglial proliferation results. An intermingling of fibers of both sources forms heavy fasciculi which run at right angles to the margin of the wound. This appearance in the phosphotungstic acid preparations suggests some alternation and intertwining of the bundles of connective tissue and neuroglia fibers, resembling a coarse, irregularly woven material. The superficial position of the connective tissue and the deeper situation of the proliferating neuroglia answer the description of old wounds of the brain as given by Penfield<sup>2</sup> and by Foerster and Penfield.<sup>3</sup> The characteristic fusiform shape of the astrocytes and the arrangement of their fibers indicate their identity with the piloid astrocytes of Penfield.<sup>4</sup>

In a study of the neuroglial reaction at the margins of recent wounds of the brain<sup>5</sup> there was found a peculiar reticular network of tissue in the interstices of which small hyperchromatic nuclei were observed. The nature of the tissue forming this mesh could not then be definitely established for it was not demonstrated in specific preparations. From the appearance of the characteristic astrocytic network of similar arrangement in a case with this long period of survival, it seems likely that the original structure was of neuroglial origin. One wonders whether the original structure was due to active regressive changes in the neuroglia consequent to the injury, or whether it was the remains of neuroglial syncytium the existence of which has been so long debated.

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2. Penfield, Wilder: *Brain* **50**:499, 1927.

3. Foerster, O., and Penfield, W.: *Brain* **53**:99, 1930.

4. Penfield, Wilder: *Neuroglia, Normal and Pathological*, in Penfield, W.: *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 455.

5. Rand, C. W., and Courville, C. B.: *Arch. Neurol. & Psychiat.* **27**:1355, 1932.

The occurrence of end-bulbs on the severed ends of nerve fibers was first described in detail by Cajal in a group of studies of experimental injuries of the brain.<sup>6</sup> These end-bulbs, recognized to be the result of regressive change rather than abortive attempts at repair, are found within a few hours after the injury, and those on the central end of the fiber may persist for months or even years. In the case under consideration, after an interval of twenty-two years, characteristic end-bulbs were not found. At the termination of the finer fibers, small irregular and at times somewhat complex expansions were observed under higher magnifications. The terminal portions of the larger fibers were slowly undergoing granular degeneration, and no end-bulbs of any sort were found. It is of interest to note in this connection that the fine fibers have been the first to reveal evidence of the formation of end-bulbs (within three hours of injury) and have been able to maintain their integrity closer to the margin of the wound. They are here discovered to retain still a residual terminal expansion, presumably a considerably modified end-bulb. This late form is probably the result of a long-continued, gradual degeneration of the original structure.

From a study of the nerve cells in the isolated cortex along the dorsal margin of the hemisphere, it has been possible to learn the fate of end-bulbs formed at the ends of fibers sectioned close to their cells of origin. In Cajal's experimental injuries of the brain, fibers sectioned in the sub-cortical white matter or in the deeper portions of the cortex underwent degenerative change in a retrograde direction to the last collateral where a residual end-bulb was formed. I have observed end-bulbs in this situation in the brain of a patient who survived a serious injury of the head for from six to seven months, so they may persist for at least this interval. After this there evidently takes place a slowly progressive disintegration of the residual segment of axon, for in the case at hand a short stub terminated just below the cell of origin in a "late corrosion point." The nerve cells in these instances retained their morphologic identity although no tigroid material could be identified in their cytoplasm, and their neurofibrillar apparatus had undergone granular disintegration.

A study of Cajal's experimental work leads one to believe that the end-bulbs and varicosities of the axis cylinders are coincidental with the myelin swellings. In a study of nerve fibers in recent injury of the human brain, at least one essential difference has been observed. Numerous myelin swellings of variable size and irregular distribution have been observed along the course of a nerve fiber in which no end-bulbs or varicosities were found to exist. Similarly, in consecutive

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6. Ramón y Cajal, S.: *Trab. d. lab. de invest. biol., Univ. de Madrid* 9:1, 39, 181 and 217, 1911.



sections stained by specific methods for myelin and for axis cylinders, in areas where end-bulbs were frequent, no myelin swellings were observed. In the case here considered, after such a long interval between the injury and death, characteristic myelin swellings were still present, while the end-bulbs and varicosities had disappeared. It seems that the swellings in the myelin sheaths occurred primarily, presumably evidence of a degenerative change in this structure, and that such swellings exerted a distorting influence on the rows of granules resulting from a breaking up of the axis cylinder.

There exists in the larger fibers a long segment of degeneration, found extending for some distance from the margin of the wound. In this zone both the myelin sheath and the axis cylinder were affected. This segment might well be designated as the "segment of late disintegration." It stands out in contrast to the finer fibers which still maintain their identity well up to the margin of the wound.

The significance of the yellowish-orange pigment contained in the macrophages scattered throughout the reticular tissue or agglutinated about the blood vessels is not entirely clear. In recent injuries their yellow color appears to be due to the presence of pigment resulting from the disintegration of red cells in the areas of local hemorrhage. While possible, it is difficult to conceive that pigment from such a source could persist for so long a time. Whether it is due to a continual process of disintegration of red cells or whether it is derived from some other source could not be ascertained.

#### SUMMARY AND CONCLUSIONS

Morphologically, crippled nerve cells may persist in the margins of wounds of the brain for many years. Nerve cells may, and usually do, persist in areas of cortex isolated by laceration or hemorrhage.

Even after a prolonged interval the larger nerve fibers continue to undergo regressive change at the margin of the wounds of the brain. Both the myelin sheaths and the axis cylinders are involved in this process of disintegration.

Characteristic end-bulbs were not observed in the case reported after an interval of twenty-two years. The finer fibers terminated in small filaments, spear-shaped or flame-shaped endings. When the corticifugal axis cylinders were cut off just below the cortex, the proximal portion had degenerated to within a few microns of the persisting nerve cell where it terminated in a late corrosion point.

Evidences of a persistent degenerative change were further indicated by the occurrence of free fat, yellow pigment and argentophilic material in wandering macrophages and the walls of the blood vessels, and by characteristic morphologic changes in the microglia in the adjacent cortex.

A solid cicatrix was not observed. In an area subjacent to the wound of entrance a combined connective tissue and neuroglial scar was found in which piloidal astrocytes played a conspicuous rôle. Elsewhere the neuroglial reaction was minimal and the connective tissue reaction entirely absent.

One peculiar behavior of connective tissue was its complete or incomplete replacement of the parenchymatous and interstitial elements in small portions of residual cortex over severely injured white substance.

The reticular tissue, observed in other instances within a few days after injury, ultimately comes to be formed of neuroglial elements, as indicated in the case under consideration.

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# ACTION OF VITAMIN D AND OF THE PARATHYROID HORMONE ON THE CALCIUM METABOLISM

AS INTERPRETED BY STUDYING THE EFFECT OF SINGLE DOSES ON THE CALCIFICATION OF DENTIN

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AND

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## INTRODUCTION

Several theories have been proposed to explain the action of vitamin D and of the parathyroid hormone on the calcium metabolism. The problem is complicated by the many seemingly paradoxical phenomena which occur when the substances are administered in different dosages. A simple example illustrates this point, namely, the ability of small amounts of vitamin D to improve the calcification of bone, whereas enormous doses not only cause a removal of calcium from the skeleton but institute pathologic calcifications in the soft tissues. It is thought that experiments utilizing single doses of these substances offer distinctive opportunities for the investigation of their action, and in this connection both Laas<sup>1</sup> and Ham<sup>2a</sup> reported that there is a latent period before the onset of pathologic calcifications when vitamin D is administered in single massive doses. Ham and Portuondo<sup>3</sup> found that the development of the pathologic calcifications was associated with the fall of the serum calcium level after hypercalcemia had been attained. They suggested that the calcifications were caused by the inability of the serum to hold in simple solution all the calcium released from the solution maintained by the parathyroid hormone when the effect of the latter had begun to diminish.

This theory of the mechanism of calcification in hypervitaminosis D depends on the acceptance of the hypothesis which pertains to the ability

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The chemical phase of this work was prepared and written by Arthur W. Ham, the histologic phase by Isaac Schour.

1. Laas, E.: *Virchows Arch. f. path. Anat.* **278**:346, 1930.

2. Ham, A. W., (a) in Cowdry, E. V.: *Special Cytology*, ed. 2, New York, Paul B. Hoeber, Inc., 1932; (b) *Arch. Path.* **14**:613, 1932; (c) *Angle Orthodontist* **2**:142, 1932.

3. Ham, A. W., and Portuondo, B. C.: *Arch. Path.* **16**:1, 1933.

of vitamin D to increase the amount or activity of the parathyroid hormone in the circulation so that this hormone in turn attracts calcium from the various tissues of the body, particularly, the bones and intestine. Thus the hypercalcemia which develops would be caused to a large extent by the increase in the amount of the fraction of the serum calcium controlled by the parathyroid hormone. If this theory is correct, there would be less calcium available for the process of normal calcification in the body during the rise in the serum calcium level following the administration of a massive dose of vitamin D, because the shift in calcium during the rise in the serum calcium level would be from the tissues toward the blood. If, on the other hand, any of the various other theories regarding the action of vitamin D is correct, such as its supposed ability to act on the intestinal epithelium so as to allow a greater absorption of calcium, or to increase the ionization of calcium, there is good reason to believe that the process of normal calcification would undergo no change except possible improvement during the rise in the serum calcium level. This study is concerned with the investigation of the process of normal calcification during the rise and fall of the serum calcium level which ensues following the administration of single doses of either vitamin D or the parathyroid hormone. Rats were used for the experiments, and the histologic studies were made on the dentin of the growing incisor. Erdheim<sup>4a</sup> justly compared the sensitivity of this tissue to the drum of a kymograph. It grows continually and rapidly enough to offer definite areas as indexes of the process of calcification on separate days and consequently appears to be a unique tissue for this type of study.

#### MATERIAL AND METHODS

This study was based on three series of experiments.

Series C consisted of eight white rats, weighing about 110 Gm., which received one dose of 0.75 cc. of ergosterol 10,000  $\times$ . The series included two rats which were killed at the beginning of the experiment. The remaining animals were killed at intervals of twenty-four, forty-eight and seventy-two hours following the administration of one dose (table 1).

Series Z consisted of twenty-nine adult male white rats which received one dose of 0.5 cc. of ergosterol 10,000  $\times$ . The series included four rats which were killed at the beginning of the experiment. The remaining animals were killed at intervals of sixteen, twenty-four, forty, forty-eight, sixty-four, seventy-two, ninety-six and one hundred and thirteen hours following the administration of one dose (table 2).

Series Pa consisted of eight white rats, weighing about 165 Gm., which received one dose of 40 units of parathyroid extract-Collip by subcutaneous injection into the back. The series included two rats which were killed at the beginning of the

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4. Erdheim: (a) Frankfurt. Ztschr. f. Path. 7:295, 1911; (b) *ibid.*, p. 175; (c) *ibid.*, p. 238.

TABLE 1.—*Serum Calcium of Eight Rats That Received One Dose of 0.75 Cc. of Ergosterol 10,000 X*

Rat	Hours After Oral Administration	Serum Calcium in Mg. per 100 Cc. of Serum
1.....	0	10.30
2.....	0	10.30
3.....	24	10.08
4.....	24	13.06
5.....	48	17.41
6.....	48	16.32
8.....	72	14.70
9.....	72	15.33

TABLE 2.—*Serum Calcium of Twenty-Nine Adult Male Rats That Received One Dose of 0.5 Cc. of Ergosterol 10,000 X*

Rat	Hours After Oral Administration	Serum Calcium in Mg. per 100 Cc. of Serum
1.....	0	9.80
2.....	0	9.60
3.....	0	10.61
4.....	0	10.94
6.....	16	11.02
7.....	16	12.96
8.....	24	12.16
9.....	24	13.68
10.....	24	13.30
11.....	40	13.02
12.....	40	13.02
13.....	40	13.65
14.....	48	13.02
15.....	48	15.54
16.....	48	15.96
17.....	64	14.91
18.....	64	15.12
19.....	64	14.70
20.....	72	16.33
21.....	72	17.07
22.....	72	12.25
23.....	96	14.55
24.....	96	16.14
25.....	96	12.62
26.....	96	12.41
27.....	113	13.79
28.....	113	13.99
29.....	113	12.98
30.....	113	14.41

TABLE 3.—*Serum Calcium of Eight Rats That Received One Dose of 40 Units of Parathyroid Extract-Collip*

Rat	Hours After Subcutaneous Injection	Serum Calcium in Mg. per 100 Cc. of Serum
1.....	0	9.880
2.....	0	9.710
3.....	18	13.390
9.....	66	10.880
10.....	66	10.490
11.....	94	10.810
12.....	94	10.600
13.....	116	10.815

experiment. The remaining animals were killed at intervals of eighteen, sixty-six, ninety-four and one hundred and sixteen hours following the administration of one dose (table 3).

The animals were fed on the stock laboratory diet. A blood calcium analysis was made of each animal at the time of death (tables 1, 2 and 3) by the Collip and Clark modification of the Tisdall-Kramer method.

A histologic study was made of one or two incisors of each animal. These teeth with their investing tissues were fixed in a 5 per cent solution of formaldehyde immediately after the animal was killed. Roentgenograms were then taken, and the teeth were washed, decalcified in 5 per cent nitric acid, dehydrated, embedded in pyroxylin (celloidin) and stained with hematoxylin and eosin. The sections were cut longitudinally or transversely and mounted in serial order. Longitudinal and transverse ground sections were made of some of the teeth of the animals in series C and Pa.

#### CHEMICAL FINDINGS

*Series C.*—In the control animals the blood calcium was normal (10.3 mg. per hundred cubic centimeters of serum). In the experimental animals the blood calcium rose to 17.41 mg. as the postoperative period increased to forty-eight hours. It declined subsequently (table 1).

*Series Z.*—In the control animals the blood calcium was normal (from 9.8 to 10.94 mg. per hundred cubic centimeters of serum). In the experimental animals the serum calcium gradually rose to 17.07 mg. as the postoperative period increased to seventy-two hours (table 2).

*Series Pa.*—In the control animals the blood calcium was normal (9.71 and 9.88 mg. per hundred cubic centimeters of serum). In the experimental animals the blood calcium rose to 13.39 mg. in the animal that lived eighteen hours after the injection. It declined toward normal subsequently (table 3).

#### HISTOLOGIC OBSERVATIONS

*Histology and Physiology of the Dentin of the Normal Rat Incisor.*—Before presenting our histologic observations on the dentin of the experimental animals, we shall consider briefly the normal physiology and histology of the dentin in the incisor of the rat.

The incisor of the rat is a tooth of continuous growth, and its weekly rate of eruption in the mature animal is about 2 mm. for the upper and about 2.8 mm. for the lower tooth. Its tissues including the dentin are, therefore, formed rapidly and in the mature animal are worn down just as rapidly at the incisal edge. The dentin grows by apposition at the pulpal surface in the form of layers which move anteriorly along the long axis of the tooth and are constantly replaced by similar layers.

Thus, in the incisor of a rat killed at a given time, a given layer of dentin is situated next to the pulp or midway in the dentin substance or near the surface next to the enamel or cementum, depending on whether this stripe of dentin was laid down approximately one, twenty or forty



days before the death of the animal. The reason for this is that the incisor of the rat renews itself about every thirty-five to forty-five days. Marshall<sup>5</sup> found that in the mature rat the dentin grew 10 microns in twenty-four hours.

The matrix of the dentin is calcified in the form of globules that are normally small and numerous and so close together that uniformly calcified tissue results. But even in normal dentin the successive layers are not equally well calcified. Well calcified layers alternate more or less regularly and rhythmically with imperfectly calcified layers, so that there arises a stratification in the dentin, especially toward the incisal edge (Schour<sup>6</sup>). There is a distinct smooth boundary between the still uncalcified matrix that was laid down last, called predentin, and the calcified matrix of the dentin (fig. 1).

#### HISTOPATHOLOGY OF THE DENTIN OF THE EXPERIMENTAL ANIMALS

*Series C.*—Significant changes were observed only in the dentin formed after the injection of ergosterol. Animals C 1 and C 2, which were controls, showed normal dentin (figs. 1 and 4). Animal C 3, which was killed twenty-four hours after the injection, showed an abnormally wide layer of predentin and prominent interglobular spaces in the dentin that was calcified last, so that the boundary between the predentin and the calcified dentin was irregular (figs. 2 and 3). On the other hand, animal C 6, which was killed forty-eight hours after the injection, showed a normal picture. Animals C 8 and C 9, which were killed seventy-two hours after the injection, generally showed the following stripes in the dentin when it was traced toward the pulp: *A*, a stripe of dentin which showed a normal reaction to the hematoxylin and eosin stain and which was situated in the part of the dentin that was laid down before the injection; *R*, a layer of dentin which took a predominately eosin stain; *F*, a layer of dentin which took a deep hematoxylin stain, and *P*, a layer of predentin which was of approximately normal width (figs. 6 and 7).

In some fields, however, animals C 8 and C 9 showed some interesting variations and disturbances in this sequence in reference to stripes *F* and *P*. Thus, figure 12 shows the more typical arrangement, as indicated in figures 6 and 7, in the dentin on the left, but in the vertical border at the right the stripe *F* is much narrower, so that stripe *P* is abnormally wide. In figure 5 somewhat similar reaction is apparent; stripe *F* is present in the form of only a very narrow band, so the

5. Marshall: J. Dent. Research 3:241, 1921.

6. Schour, I., in Cowdry, E. V.: Special Cytology, ed. 2, New York, Paul B. Hoeber, Inc., 1932.

layer of predentin is wider than normal. Figure 4 shows a corresponding section in the control rat C 1. Figure 11 shows an abnormally wide layer of predentin (*P*).

*Series Z.*—Significant changes were observed only in the dentin that was formed during the time that elapsed following the injection of ergosterol.

Animals Z 1, 2, 3 and 4, which were controls, showed normal dentin,

The changes in the experimental animals varied according to the length of postoperative life.

Practically all the experimental animals of this series showed a stripe of light, eosin-staining dentin located in a position corresponding with that of the matrix of the dentin which was laid down soon after the time of the injection. This stripe was more or less constant in width except in the most anterior portion of the teeth of the animals of longest postoperative life. In addition, in the animals that lived forty-eight hours or more after the injection, the eosin-staining stripe was followed pulpally by a stripe of deep hematoxylin-staining dentin that increased in width as the postoperative hours of life increased in number.

Thus, figure 8, illustrating the formation of dentin in animal Z 15, which lived forty-eight hours after the injection, showed the following layers of dentin when they were traced toward the pulp: *A*, normally staining dentin of preoperative history; *R*, a light stripe in which eosin predominated, and *F*, a stripe in which hematoxylin predominated. In figure 9, which illustrates the dentin in animal Z 27, which was killed one hundred and thirteen hours after the injection, one finds a similar arrangement, except for layer *R*, which was considerably wider.

*Series Pa.*—Significant changes were observed in the dentin formed after the injection of parathyroid extract-Collip. On the whole, the reaction of the dentin in this series was quite similar to that in series C. Animals Pa 1 and Pa 2, which were controls, showed normal dentin.

Animal Pa 3, which was killed eighteen hours after the injection, showed changes in the dentin formed last similar to those found in animal C 3 (figs. 2 and 3). Animal Pa 9, which was killed sixty-six hours after the injection, showed a normal picture similar to that of animal C 6. Animals Pa 10, 11, 12 and 13 showed the following orderly succession of stripes when they were traced toward the pulp: *A*, dentin which showed a normal reaction to the hematoxylin and eosin stain and which represents the dentin that was calcified before the injection; *R*, a layer of dentin which took a light eosin stain of fairly constant width, about 14 microns; *F*, a layer of dentin which took a deep hematoxylin stain and increased in width with an increase in the number of postoperative hours (about 40 microns in animal Pa 10 and about 70 microns in animal Pa 12), and *P*, a layer of predentin of normal width (fig. 10).

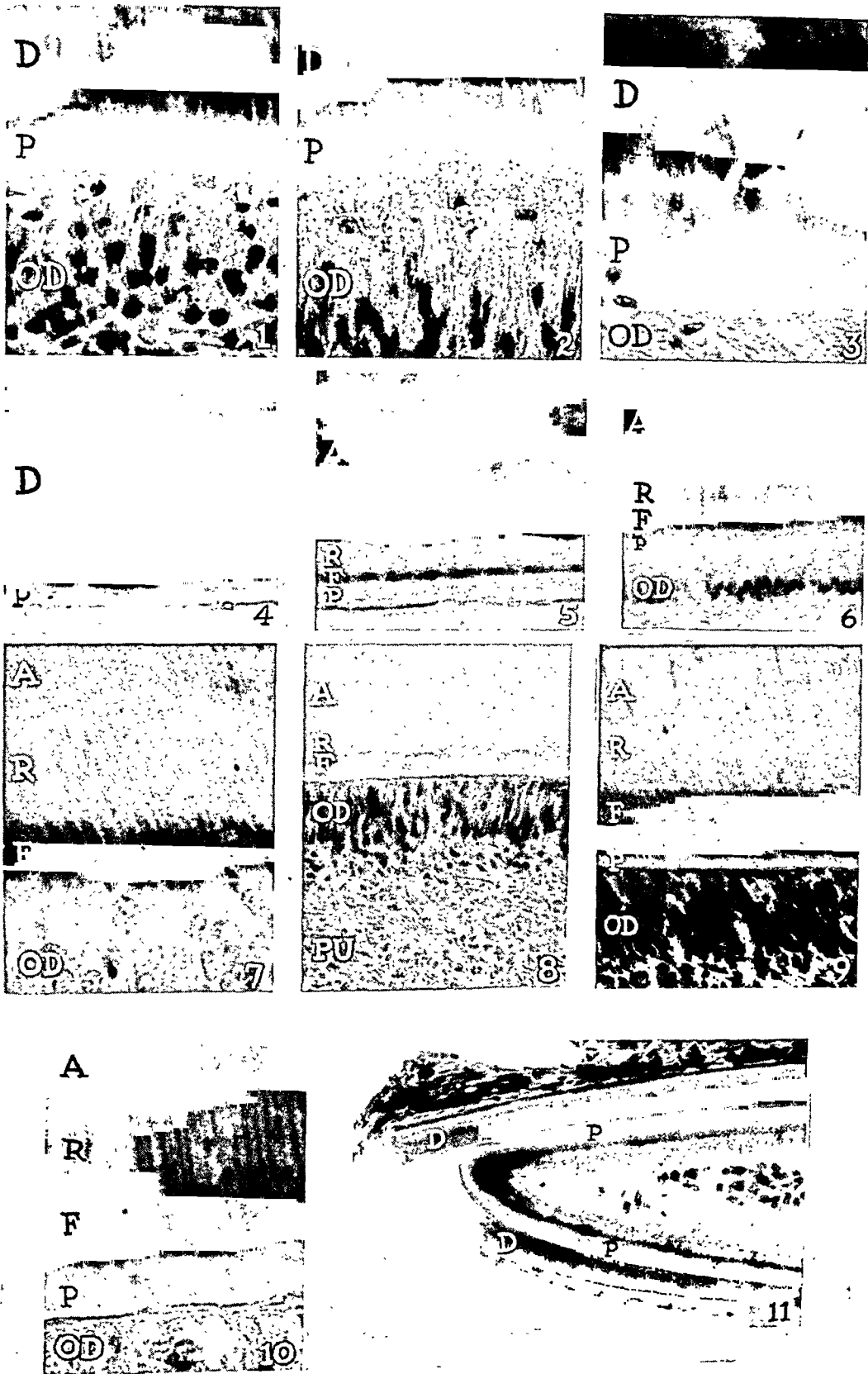


Plate 1

## EXPLANATION OF PLATE 1

Fig. 1.—Photomicrograph of a section of labial dentin of the midregion of the upper incisor of control rat C 2;  $\times 490$ . *D*, calcified dentin; *OD*, odontoblasts; *P*, predentin.

Fig. 2.—Photomicrograph of a section of labial dentin of the midregion of the upper incisor of rat C 3, which was given a single dose of 0.75 cc. of ergosterol 10,000  $\times$  and killed twenty-four hours later;  $\times 490$ . Note the wide layer of predentin, *P*, and compare with figure 1; *D*, dentin; *OD*, odontoblasts.

Fig. 3.—Photomicrograph of a section of labial dentin of the posterior region of the upper incisor of the same rat as in figure 2;  $\times 490$ . Note the isolated globules of dentin, *D*, and the irregular boundary between the latter and the predentin, *P*; *OD*, odontoblasts.

Fig. 4.—Photomicrograph of a section of lingual dentin of the posterior region of the upper incisor of control rat C 1;  $\times 490$ . *D*, dentin; *P*, predentin.

Fig. 5.—Photomicrograph of a section of lingual dentin of the posterior region of the upper incisor of rat C 8, which was given one dose of 0.75 cc. of ergosterol 10,000  $\times$  and killed seventy-two hours later;  $\times 490$ . *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium and showing practically no calcification; *F*, dentin laid down during the fall of the blood calcium; *P*, predentin. Compare with figure 4.

Fig. 6.—Photomicrograph of a section of labial dentin of the upper incisor of the same animal as in figure 5;  $\times 244$ . *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium and showing poor calcification; *F*, dentin laid down during the fall of the blood calcium; *OD*, odontoblasts.

Fig. 7.—Photomicrograph of a section of labial dentin of the upper incisor of rat C 9, which was given one dose of 0.75 cc. of ergosterol 10,000  $\times$  and killed seventy-two hours later;  $\times 490$ . *A*, dentin laid down before the experiment; *R*, dentin laid down during the rise of the calcium curve and showing imperfect calcification; *F*, dentin laid down during the fall of the blood calcium and showing better calcification than in *A*; *OD*, odontoblasts.

Fig. 8.—Photomicrograph of a section of labial dentin of the midregion of the lower incisor of rat Z 15, which was given one dose of 0.5 cc. of ergosterol 10,000  $\times$  and killed forty-eight hours later;  $\times 244$ . *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium; *F*, dentin laid down during the fall of the blood calcium; *OD*, odontoblasts; *PU*, pulp.

Fig. 9.—Photomicrograph of a section of labial dentin of the lower incisor of rat Z 27, which was given 0.5 cc. of ergosterol 10,000  $\times$  and was killed one hundred and thirteen hours later;  $\times 244$ . *A*, dentin laid down and calcified before the experiment; *R*, dentin calcified during the rise of the blood calcium; *F*, dentin calcified during the fall of the blood calcium and showing more intense calcification than *A*; *OD*, odontoblasts; *P*, predentin.

Fig. 10.—Photomicrograph of a section of labial dentin of the upper incisor of rat Pa 10, which was given one dose of 40 units of parathyroid extract-Collip and killed sixty-six hours later;  $\times 490$ . *A*, dentin laid down and calcified before the experiment; *R*, dentin showing poorer calcification than *A*; *F*, dentin showing better calcification than *R*; *P*, predentin.

Fig. 11.—Photomicrograph of a section of lingual dentin of the same rat as in figures 5 and 6;  $\times 244$ . Note that the fracture that occurred during the dissection extends only through the calcified dentin, *D*, while the predentin, *P*, is bent. The predentin is wider than normal.

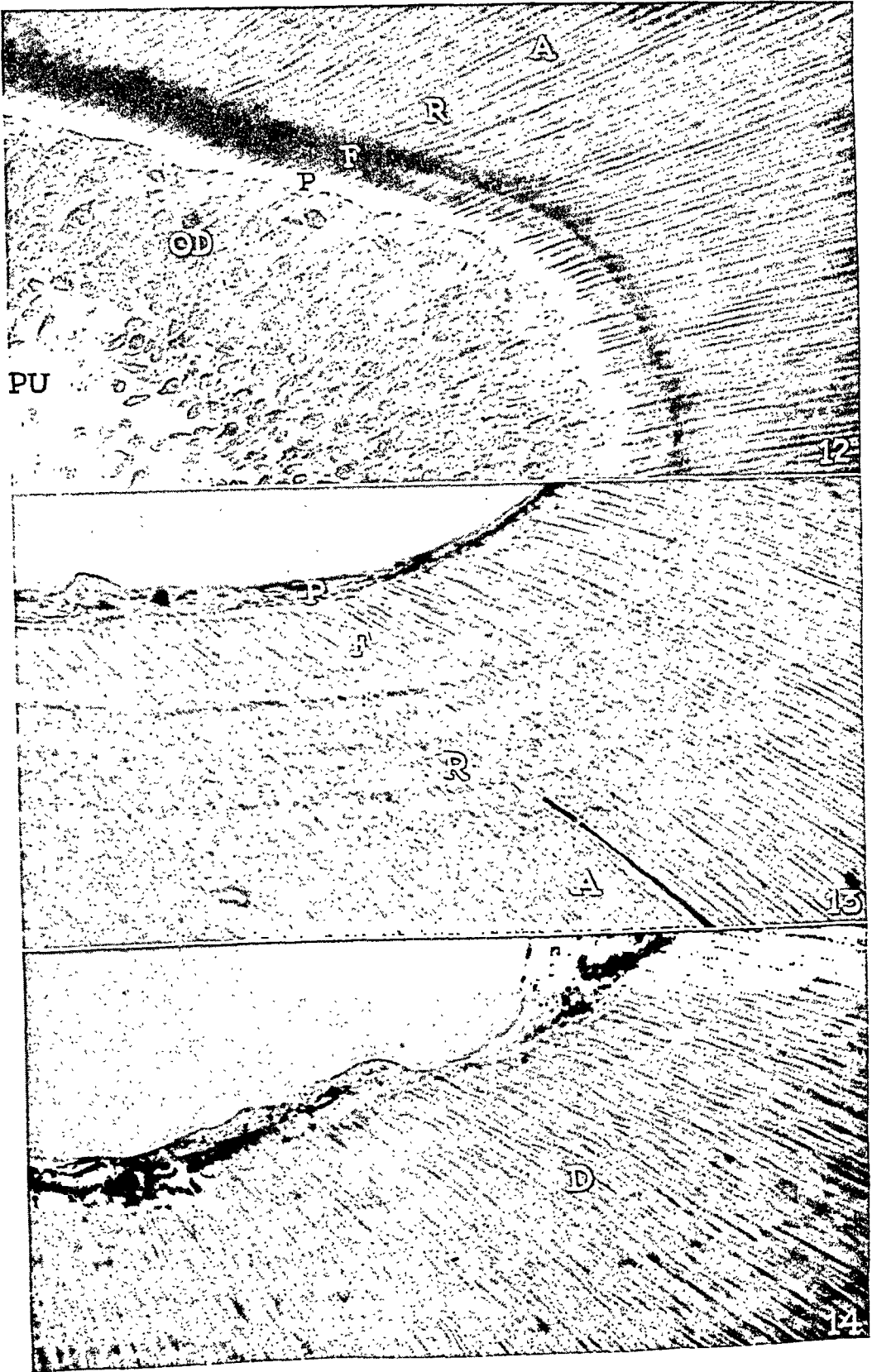


Plate 2

## EXPLANATION OF PLATE 2

Fig. 12.—Photomicrograph of a transverse section of dentin of the upper incisor of the same rat as in figures 5 and 6;  $\times 490$ . *A*, dentin laid down and calcified before the experiment; *R*, dentin calcified during the rise of the blood calcium; *F*, dentin laid down during the fall of the blood calcium; *P*, predentin; *OD*, odontoblasts; *PU*, pulp. Note that the dentin layer, *F*, takes a more intensive stain than *A*.

Fig. 13.—Photomicrograph of a ground transverse section of dentin of the upper incisor of rat C 9, which had same experimental history as animal C 8 of figure 12;  $\times 490$ . *A*, dentin laid down previous to the experiment; *R*, dentin laid down during the rise of the blood calcium; *F*, dentin laid down during the fall of the blood calcium.

Fig. 14.—Photomicrograph of a ground transverse section of dentin of the upper incisor of control rat C 1;  $\times 490$ . Note normal calcification of dentin, *D*, and compare with figure 13.

*Summary of the Histologic Reaction.*—The histologic picture can be seen best in the experimental animals of the three series which were killed last, because these present a more complete record. Three principal types of stripes can be distinguished in the dentin of their incisors:

1. A stripe of dentin which reacted normally to the hematoxylin and eosin stain and which represents the preoperative dentin. This stripe naturally is by far the widest, as the animals were allowed to live only a relatively short time after the operation (from sixteen to one hundred and sixteen hours).

2. A stripe of dentin which took a light eosin stain and which represents the dentin that was imperfectly calcified during the rise of the blood calcium curve. This stripe was of constant width except in some of the animals of longer postoperative life, in which its most anterior portion was narrower.

3. A stripe of dentin which took a deep hematoxylin stain and which represents the dentin that was well or excessively calcified during the decline of the blood calcium curve. Its width varies directly with the length of postoperative life.

There were some incidental variations from this pattern in individual cases. Thus, in the animals of series C which had a longer postoperative life, the dentin showed localized disturbances. This may be associated with the higher dosage of ergosterol and the younger age of the animals.

#### COMMENT

*Preliminary Histologic and Chemical Studies Made Independently.*—In order to avoid the influence that the knowledge of the chemical history might have on the study of the histologic observations, the latter were first made by one of us (I. Schour) without knowledge of the blood calcium curves. The histologic study led to the conclusion that the dentin laid down soon after the injection was imperfectly calcified, while the dentin laid down later was excessively calcified. This conclusion coincided closely with the expectations that were deduced independently by A. W. Ham.

*The Validity of Hematoxylin as an Indicator of Calcification.*—Although we are fully aware of the limitations pointed out by Cameron<sup>7</sup> and Hagens,<sup>8</sup> in judging the state of calcification by means of hematoxylin and eosin staining, we regard the latter as a workable and dependable method for dentin for the following reasons:

1. In decalcified sections the calcified matrix of the dentin takes the hematoxylin and the uncalcified matrix takes the eosin stain, as is

7. Cameron, J. R.: J. Path. & Bact. **33**:929, 1930.

8. Hagens, E. W.: Arch. Otolaryng. **13**:824, 1931.

borne out by roentgenograms. Radiopaque dentin takes the hematoxylin and radiolucent dentin takes the eosin stain in histologic sections (Toyofuku,<sup>9</sup> Schour and van Dyke<sup>10</sup>).

2. When a fracture occurs in the dentin tissue, the former is seen in histologic sections to be limited to the dentin which takes the hematoxylin stain and does not extend into the dentin which stains with eosin. Figure 11 shows a fracture that occurred during the dissection of the upper left incisor of animal C 8. The fracture extends through the dentin which took the hematoxylin stain. In the dentin which took the eosin stain there is no break in continuity but merely a bending at an angle of about 130 degrees. The fracture occurred only in the part of the dentin which was calcified. Erdheim<sup>11</sup> reported fractures in parathyroidectomized rats that extended in the enamel and in the blue-staining dentin, while "the calcium-free red-staining dentin, because it was soft and yielding, remained intact." He also reported similar observations in ribs that fractured spontaneously following parathyroidectomy.

3. Erdheim<sup>12</sup> observed that, in the presence of suppuration, dentin which stained with eosin was readily invaded by pus cells, while the latter did not penetrate hematoxylin-staining dentin which was entirely surrounded by pus. He associated the lack of penetration in the blue-staining dentin with its hardness.

4. Ham<sup>2b</sup> observed that in pathologic calcifications produced by hypervitaminosis D the media of the coronary vessels which stained deeply with hematoxylin showed a greatly increased mineral content in the incinerated sections. He also reported that the observation of calcareous deposits in the aorta, based on the hematoxylin reaction, was substantiated in animals of similar history which were given an injection of alizarin red, and that these showed clearly, in the gross, pinkish areas in the wall of the vessel.

5. A study of ground sections of corresponding fields revealed that the stripes which in the ground sections showed good homogeneous calcification, as evidenced by the absence of interglobular spaces, corresponded to the stripes in the decalcified sections which took more of the hematoxylin stain (figs. 13 and 14).

For the reasons just stated, we feel justified in interpreting in decalcified sections a dentin stripe which stains only lightly with hematoxylin as dentin that is poorly calcified. By the same token, a stripe which stains deeply with hematoxylin represents dentin that is well or excessively calcified, depending on the intensity of its staining reaction.

*Effect of the Rise in Blood Calcium.*—The fact that the width of the eosin-staining stripe laid down during the rise of the blood calcium

9. Toyofuku: Frankfurt. Ztschr. f. Path. 7:249, 1911.

10. Schour, I., and van Dyke, H. B.: Am. J. Anat. 50:397, 1932.



level diminished in some of the animals which had a longer postoperative life suggests a partial recovery of the defect in the dentin that was laid down soon after the injection. Thus, during the fall of the blood calcium there is, as a rule, not only an improvement in the calcification of the dentin laid down at the time, but there may be, in addition, secondary calcification of the dentin previously imperfectly calcified.

*Sensitivity of the Dentin Reaction.*—Erdheim's statement regarding the delicacy of the reaction of dentin to changes in the calcium metabolism is fully confirmed by the observations in this study and by the unreported observations in a study made by one of us (I. Schour) in collaboration with McJunkin, Tweedy and Breuhaus.<sup>11</sup> The histologic study of the dentin of the incisor of the rat is found to afford much better advantages than does the study of bone in mirroring the processes that take place in the calcium reservoir. The dentin yields a delicate and a chronological record of the changes that occur during the entire duration of the experiment, provided the experiment does not last more than from four to five weeks. This suggests the possibility of using this tissue as an indicator in a biologic assay of substances that affect the calcium metabolism. The delicacy of the dentin reaction is comparable to that of the feather test used as an indicator for the female sex hormone.

#### THE ACTION OF THE PARATHYROID HORMONE

The literature on the extraordinarily complicated field of the calcium metabolism and the action of the parathyroid hormone has recently been comprehensively reviewed by Thomson and Collip.<sup>12</sup> They suggested that the hormone acts either (1) by forming very slowly, even in the presence of excess calcium, a calcium compound of parathyroid hormone, (2) by stimulating the production of an unknown calcium-binding substance, or (3) by acting primarily on the bones, with a resulting active liberation of calcium from the latter. The first two theories presume that the hormone increases the attraction of the blood for calcium, so the withdrawal of calcium from the bones would necessarily be of a passive nature. The last theory suggests direct action of the hormone not on blood but rather on bone, which results in the active liberation of calcium from the latter. Thomson and Collip<sup>12</sup> appeared to favor the latter theory and suggested that it is in accord with the histologic evidence which, they believed, indicates active rather than passive liberation of calcium from bone.

In the development of this theory it is obvious that a considerable amount of faith has been placed in the hypothesis which postulates that

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11. McJunkin, F. A.; Tweedy, W. R., and Breuhaus, H. C.: Arch. Path. **14**:649, 1932.

12. Thomson, D. R., and Collip, J. B.: Physiol. Rev. **12**:309, 1932.

the osteoclast is an active agent in the resorption of bone. It should be remembered that this theory regarding the action of osteoclasts arose when it was believed that both the deposition and the removal of calcium was accomplished by means of specific cellular activity in bone. Recent studies in the mechanism of calcification have established that the process is of a physicochemical nature and that the cells are only indirectly concerned. In the light of this knowledge, the necessity for specific cellular activity in the process of resorption should be questioned, particularly as the evidence concerning the ability of osteoclasts in this respect has never been very convincing (Howell,<sup>13</sup> Arey<sup>14</sup>). Recently, Ham<sup>2a, c</sup> interpreted the osteoclast as the differentiation product of the osteogenic cell in the presence of a foreign body type of stimulus. He believed that osteogenic cells are differentiated into giant cells (osteoclasts) in the presence of breaking-down bone or cartilage in much the same manner that foreign body giant cells arise when fat is breaking down in fat tissue. In other words, he regarded them as arising primarily as the result of the disintegration of bone and cartilage. After their formation they may cause indentations (Howship's lacunae) on the surface of the bone because of the proximity of their cytoplasm to the calcified matrix. The stimulus for their origin, however, is thought to be not a need for the removal of calcium but a foreign body type of stimulus arising because of the disintegration of bone and cartilage that is already progressing.

If osteoclasts are regarded as the effect of the resorption of bone rather than as its cause, their presence in bones after the administration of parathyroid hormone offers no evidence of the direct action of the hormone on osteoclasts. Furthermore, the results of our experiments on dentin tend to disprove the theory concerning direct action on bone, as the calcification of dentin was interfered with despite the absence of osteoclasts in this region. It therefore appears that our results can best be explained by the theory which postulates that the hormone controls, directly or indirectly, a fraction of the serum calcium. Examination of the evidence for and against this theory would involve the consideration of an immense amount of controversial literature. It is discussed in detail in the reviews of Peters and Van Slyke,<sup>15</sup> Thomson and Collip<sup>12</sup> and Barr.<sup>16</sup> It is thought, however, that in the application of this theory sufficient care has not been taken to distinguish the changes which occur during the period of rise in the serum calcium level following a single dose of the hormone from those which occur as the serum

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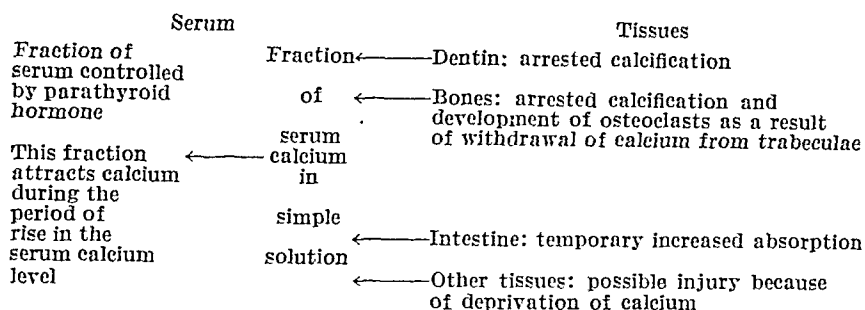
13. Howell: *J. Morphol.* **4**:117, 1890.

14. Arey, L. B.: *Am. J. Anat.* **26**:315, 1920.

15. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, Williams & Wilkins Company, 1931.

16. Barr, D. P.: *Physiol. Rev.* **12**:593, 1932.

calcium level recedes. If the parathyroid hormone acts by influencing a certain fraction of the serum calcium, a large injection of the hormone into an animal would result in calcium being attracted to the blood and, more specifically, to the fraction of the serum controlled by the hormone. The shift in calcium during the period of rise in the serum calcium level following a single dose of parathyroid hormone might be diagrammatically represented as follows:



The evidence at hand which points to changes in the tissues as postulated in this diagram may be briefly summarized. Our experiments with dentin indicate that its calcification is arrested during the upswing of the serum calcium curve. Numerous investigators have shown that the bones lose calcium after administration of the parathyroid hormone (Hunter and Aub,<sup>17</sup> Hunter,<sup>18</sup> Bauer, Aub and Albright,<sup>19</sup> Stewart and Percival<sup>20</sup> and Caven and Taylor<sup>21</sup>). Collip showed that the hypercalcemic effects of the administration of the parathyroid hormone are intensified by the ingestion of calcium, a fact which would indicate increased absorption during the period of rise in the serum calcium level. The calcium content of the tissues during the period of rise in the serum calcium level can at present be only a matter of speculation. There is, however, some indirect evidence which suggests that the tissues may be injured during the period of rise in the serum calcium curve by virtue of the abstraction of calcium from them. In this connection, McJunkin, Tweedy and Breuhaus<sup>11</sup> found that injections of the hormone into the renal parenchyma caused necrosis of the tissue.

After hypercalcemia has been attained by the administration of a single dose of the hormone the calcium level begins to fall. This phenomenon may be explained by considering that the administered hormone is being eliminated, broken down or affected in some manner so that it no longer retains its former ability to attract or hold calcium.

17. Hunter, D., and Aub, J. C.: *Quart. J. Med.* **20**:123, 1927.

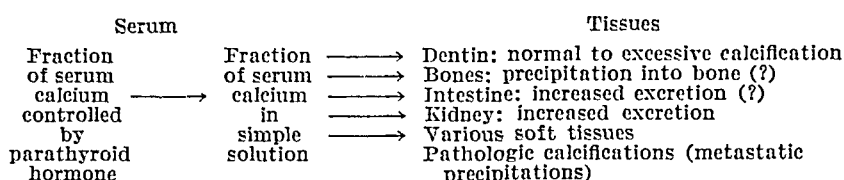
18. Hunter, D.: *Lancet* **1**:897, 947 and 999, 1930.

19. Bauer, W.; Aub, J. C., and Albright, F.: *J. Exper. Med.* **49**:145, 1929.

20. Stewart, C. P., and Percival, G. H.: *Biochem. J.* **21**:301, 1927.

21. Caven and Taylor, cited by Taylor, Weld, Branion and Kay.<sup>23</sup>

The calcium, formerly held in solution by the effect of the hormone, would then be liberated. This phenomenon would institute a shift in the equilibrium in the direction opposite to that which occurred during the period of rise, and in this phase of the process calcium might be expected to be deposited in the dentin and bones and to be excreted. There is another possibility to be considered, namely, that the release of calcium from the solution maintained by the parathyroid hormone might introduce more calcium into simple solution than could be retained without the occurrence of precipitation (Ham,<sup>2b</sup> Ham and Portuondo<sup>3</sup>). The shift in calcium which probably occurs during the fall in the serum calcium level following the hypercalcemia attained by a single dose of parathyroid hormone might be diagrammatically represented as follows:



The evidence to support this conception of the shift in calcium during the fall in the serum calcium curve is incomplete. Our work demonstrated that the calcification of dentin varied from normal to excessive during this phase. In this connection it should be again pointed out that the dentin offers a much more accurate index of the day by day process of calcification than do the bones. It is difficult to determine what picture in bone is caused by the shift in calcium during the downswing of the curve. The dentin, on the other hand, provides a new stripe of tissue for each successive day's study. The evidence for increased secretion by way of the intestine in this phase of the serum calcium curve is not yet clearcut, because most experiments in this field have been performed by administering several doses of the hormone. There is, however, good evidence to indicate that the precipitations into the soft tissues occur in this particular phase. It was shown by McLeod and Taylor<sup>22</sup> that the urgent symptoms of hyperparathyroidism made their appearance as the serum calcium curve showed a decided fall from its previous high level. Thomson and Collip<sup>12</sup> stated that "urgent symptoms of distress begin to appear at about the time when the serum calcium curve turns downward." Ham and Portuondo<sup>3</sup> showed that the calcifications of hypervitaminosis D occur during the period of fall of the serum calcium curve following a single dose of the vitamin. Thus there appears to be reasonably good evidence to suppose that the beginning of the fall in the serum calcium level coincides with a shift in calcium from the parathyroid-controlled fraction to one which must be

22. McLeod and Taylor: Tr. Roy. Soc. Canada (Sect. 5), 1925, p. 27.

retained in simple solution; and, furthermore, it appears that if the amount of calcium released is sufficiently great, precipitations will occur into the soft tissues.

#### ACTION OF VITAMIN D

Taylor, Weld, Branion and Kay<sup>23</sup> recently considered in detail the literature which pertains to the problem of the interrelationship of vitamin D and the parathyroid hormone. They submitted important evidence to indicate that large doses of vitamin D exert their effects through the parathyroid mechanism. Thomson and Collip<sup>12</sup> also discussed this problem in detail. The chief difficulty in the performance of crucial experiments in this field is the possibility of aberrant parathyroid tissue. Other theories of the action of vitamin D are not so clearcut as this one, but in general they postulate that the administration of vitamin D allows an improved absorption or utilization of calcium on the part of the affected animal. There are, of course, numerous examples in which the administration of vitamin D resulted not in an improved calcification of bone but in demineralization (Kreitmair and Hintzelmann,<sup>24</sup> Baumgartner, King and Page<sup>25</sup> and others). Grauer<sup>26</sup> reported on the development of osteitis fibrosa cystica following excessive and prolonged administration of vitamin D. In our experiments it was found that the calcification of dentin was arrested for a time following the administration of a single large dose of activated ergosterol. It is difficult to explain our results by any theory which postulates that vitamin D acts by simply increasing the absorption or ionization of calcium. On the other hand, our results may be explained readily if vitamin D is thought to act through the parathyroid mechanism, but only if the parathyroid hormone is thought to act on the blood by controlling a fraction of the serum calcium. Thus, by reference to the diagrams formulated to show the action of the parathyroid hormone, the calcification of dentin in relation to the serum calcium curve after the administration of vitamin D may be readily understood.

These experiments, it is thought, offer some evidence that vitamin D acts through the parathyroid mechanism. They furthermore indicate that the parathyroid hormone acts on the blood rather than on the bones. They offer evidence that, following a single dose of either substance, a shift in calcium occurs toward the blood while the serum calcium level is rising and toward the tissues while the level is falling. This

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23. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: *Canad. M. A. J.* **24**:763, 1931.

24. Kreitmair, H., and Hintzelmann, U.: *Arch. f. exper. Path. u. Pharmacol.* **137**:203, 1928.

25. Baumgartner, L.; King, E. J., and Page, I. H.: *Biochem. Ztschr.* **213**:170, 1929.

26. Grauer, R. C.: *Proc. Soc. Exper. Biol. & Med.* **29**:466, 1932.

theory was utilized by Ham and Portuondo to explain the mechanism of certain metastatic calcifications, and its application allows many seemingly paradoxical phenomena encountered in studies on calcium metabolism to be explained. Confusion often arises because of the failure of investigators to appreciate the difference in the state of the serum calcium under conditions of a rising curve in contrast to conditions during a falling curve.

#### SUMMARY

Since the dentin of the incisor of the rat is apposed day by day, it was selected as an index of the normal calcification process as affected by the administration of single massive doses of either vitamin D or the parathyroid hormone.

Single massive doses of either substance resulted in the formation of, first, a stripe of dentin which was imperfectly calcified and, second, a stripe of dentin which was normally or excessively calcified.

The poorly calcified dentin was found to represent the area calcified while the serum calcium level was rising, and the well calcified stripe was found to represent that calcified while the serum calcium level was falling, after the attainment of a hypercalcemia.

As no osteoclasts were found in the dentin, the action of the parathyroid hormone in preventing calcification for a time did not appear to depend on its supposed ability to stimulate the active liberation of calcium from bone.

The observations could be explained by the theory which postulates that the parathyroid hormone controls a fraction of the serum calcium.

The results with vitamin D could not easily be explained by any theory which postulates that the vitamin acts by simply increasing the absorption or ionization of calcium.

The results could be explained by the theory which postulates that vitamin D acts through the parathyroid mechanism, provided the shift in calcium during the upswing of the curve is considered to be toward the blood and, during the downswing of the curve, from the blood to the bones, dentin, soft tissues and intestine.

# SOME ABNORMALITIES IN RATS SUBSISTING ON DIETS POOR IN MINERAL NUTRIENTS

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During a study of the effects of diets low in salt on the rate of growth of rats, and on the proportion of mineral constituents deposited in their bones, it was noticed that the eyes of a number of the animals protruded abnormally. The proportion of inorganic matter in the skulls of all of the animals reared on these diets was so small that the bony tissue was soft to the touch; furthermore, the spines showed several curvatures, the legs were bowed and all of the bones were spongy. Various degrees of anemia were observed. The eyes themselves, although they appeared to be unusually large and distended with fluid, on histologic examination showed no signs of tissue damage from intra-ocular pressure, nor did the ocular muscles reveal any abnormalities. The protrusion of the eyes was not continuous; it was most marked when the animal was active, but occasionally disappeared entirely.

The effects of the realimentation of these animals with ample amounts of calcium salts or with an adequate salt mixture have been investigated.

## EXPERIMENTAL METHODS

The basal ration was composed of: casein, 18 per cent; butter fat, 9 per cent; lard, 20 per cent and dried yeast, 8 per cent; the remaining 45 per cent of the ration consisted of starch. The experimental diets were made by supplying from 0.5 to 4 per cent of different salt mixtures in place of an equivalent weight of starch. In all cases 10 drops of cod liver oil was administered daily, and distilled water was furnished ad libitum. When Sure's or McCollum's salt mixtures supplied the inorganic requirements, iodine was added to the water once weekly.

The ingredients of the basal ration, exclusive of the salt mixture, furnished 1.28 Gm. of ash per hundred grams of food; of this, 0.023 Gm. consisted of calcium and 0.245 Gm. of phosphorus. Rats restricted to such a diet died in about forty days. Male albino rats, 21 days of age, of from 40 to 50 Gm. in weight, were selected for all experiments.

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From the Department of Surgery, Section of Ophthalmology, Yale School of Medicine, and the Biochemical Laboratory of the Connecticut Agricultural Experiment Station.

A part of the expense of this investigation was borne by the Carnegie Institution of Washington.

A group of seventeen rats was fed on a diet that contained 0.5 per cent of Sure's salt mixture<sup>1</sup> and 0.28 per cent of sodium chloride. The calcium and phosphorus contents of this diet were respectively 0.063 and 0.305 Gm. per hundred grams of food. At the expiration of sixty days the femurs of nine of these rats were analyzed for ash, and the hemoglobin content of the blood was determined by a modification of the Cohen-Smith method.<sup>2</sup> The remaining eight rats then received a diet that contained 4 per cent of Osborne-Mendel salt mixture;<sup>3</sup> four rats were killed after thirty days and four after sixty days on this diet and were examined similarly.

A second group of seventeen rats was fed a diet that contained McCollum's salts<sup>4</sup> for sixty days, when nine were killed and examined as in the first group. To ascertain the effect of an adequate supply of calcium on the hemoglobin content of the blood, on the deposition of bone ash, and on the general condition of the animals, calcium carbonate was added to the diet of the remaining eight rats to make the calcium content of the ration equivalent to that of the ration in which 4 per cent of Osborne-Mendel salts had been included. Four animals received this ration for thirty and four for sixty days before analyses were made.

X-ray photographs were taken of four animals from each of these groups after thirty and sixty days on the diets low in salt, and also after thirty days of realimentation with a complete salt mixture or with calcium carbonate.

1. Sure, B.: *J. Biol. Chem.* **58**: 681, 1924. The composition of this salt mixture is:

	Gm.
Sodium chloride .....	0.2022
Anhydrous magnesium sulphate.....	0.3117
Crystallized disodium hydrogen phosphate.....	0.5265
Dipotassium hydrogen phosphate.....	1.1158
Crystallized dicalcium phosphate (calcium hydrogen phosphate)	1.1165
Calcium lactate .....	0.2896
Ferric citrate .....	0.1385

2. Cohen, B., and Smith, A. H.: *J. Biol. Chem.* **39**: 489, 1919.

3. Osborne, T. B., and Mendel, L. B.: *J. Biol. Chem.* **37**: 557, 1919. The composition of this salt is:

	Gm.		Gm.
Calcium carbonate .....	134.8	Citric acid plus water.....	111.1
Magnesium carbonate .....	24.2	Crystallized iron citrate.....	6.34
Sodium carbonate .....	34.2	Potassium iodide .....	0.020
Potassium carbonate .....	141.3	Manganous sulphate .....	0.079
Phosphoric acid, U. S. P.....	103.2	Sodium fluoride.....	0.248
Hydrochloric acid .....	53.4	Potassium aluminum sulphate	0.0245
Sulphuric acid .....	9.2		

4. McCollum, E. V.; Rask, O. S., and Becker, J. E.: *J. Biol. Chem.* **77**: 753, 1928. The composition of this salt is:

	Gm.
Sodium chloride .....	146.0
Anhydrous magnesium sulphate.....	225.0
Sodium biphosphate, U. S. P. (sodium dihydrogen phosphate) plus water.....	293.0
Dipotassium hydrogen phosphate.....	805.0
Monobasic calcium phosphate (tetrahydrogen calcium phos- phate) plus water.....	456.0
Ferric citrate .....	100.0
Calcium lactate .....	1,098.5



For purposes of control, nine animals were fed a diet that contained 4 per cent of Osborne-Mendel salts; five of these were killed at ninety days and four at one hundred and twenty days. In addition, ten rats were fed a diet that contained 0.5 per cent of McCollum's salts and 1 per cent of calcium carbonate; five of these were killed after ninety and five after one hundred and twenty days. The hemoglobin content of the blood and the ash content of the femurs were determined. X-ray photographs of two animals from each control group were taken after sixty and ninety days on the diets.

## RESULTS

Of the 34 rats fed the diets low in salt, 11 developed markedly protruding eyes; 11 developed eyes which protruded slightly; 4 developed eyes with bloody rings around them; 2 developed nervous spasms characterized by "screaming fits" in which the animals bit their tails, and 7 lost hair symmetrically from the rump and, in some cases, from the abdomen as well.

A histologic study was made of the ocular tissue and its adnexa. No definite changes were noted in the structure of the eyeball, or in the muscles that control the movements of the eye. The portion of the orbital tissue consisting of the harderian gland and of fat was normal. No signs of increased intra-ocular pressure were observed, nor was there any invasion of the ocular muscles by mononuclear cells as described by McCool and Naffziger<sup>5</sup> for exophthalmos in man.

Neither the pituitary nor the suprarenal glands revealed definite abnormalities; the thyroid gland, however, presented certain striking changes which are now under investigation.

The hemoglobin content of the blood, the ash content of the femurs and the A/R ratios (ratio of inorganic matter in the bones to the organic matter), as well as the gross abnormalities observed, are shown in the table.

Rats that had subsisted on diets low in salt for sixty days exhibited varying degrees of anemia as judged by the hemoglobin content of the blood. Realimentation of these rats, either by an increase in the total salts of the diet or by an increase in its calcium content, in every case resulted in an increased hemoglobin content of the blood. The level of hemoglobin in the blood was, however, never as high as that in the control rats of the same age.

The inorganic matter in the bones of rats fed diets low in salt for sixty days was greatly reduced. The feeding of adequate amounts, either of a mixture of salts or of calcium carbonate, resulted in a greater

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5. McCool, J. L., and Naffziger, H. C.: *Tr. Am. Ophth. Soc.* **30**: 103, 1932.

*Effects of Diets of Varied Inorganic Salt Content on the Blood and Bones and Also on the General Appearance of Rats*

Salts in Diet, per Cent	Duration of Feeding, Days	Rat Number	Hemo- globin in 100 Cc. Blood, Gm.	Ash of Dry Fat-Free Bones, per Cent	A/R	Gross Abnormalities During First 60 Days of Experiment
0.5 Sure's salts + 0.28 sodium chloride	60	C 4764	4.8	36.1	0.57	Bloody ring around one eye
	60	C 4767	11.2	43.1	0.76	Bloody ring around one eye
	60	C 4770	13.1	44.2	0.79	
	60	C 4772	9.1	46.1	0.91	
	60	C 4777	5.0	41.4	0.70	Slight protrusion of eyes
	60	C 4779	12.3	44.5	0.80	Slight protrusion of eyes
	60	C 4781	9.7	39.5	0.65	Swollen lids and closed eyes
	60	C 4787	10.5	39.7	0.66	Slight protrusion of eyes; deplilation
	60	C 4789	8.5	34.9	0.54	
Average.....			9.4	42.2	0.74	
0.5 McCollum's salts	60	C 4801	9.8	49.3	0.97	Protrusion of eyes
	60	C 4802	11.5	....	....	Slight protrusion of eyes; "screaming fits"; depilation
	60	C 4803	10.9	47.1	0.89	
	60	C 4806	9.1	47.2	0.89	
	60	C 4808	11.5	45.7	0.84	Slight protrusion of eyes
	60	C 4812	11.7	41.8	0.72	Slight protrusion of eyes
	60	C 4815	6.1	46.6	0.87	Protrusion of eyes
	60	C 4825	4.5	38.8	0.63	Slight protrusion of eyes; nervousness; depilation
	60	C 4826	10.9	....	....	Protrusion of eyes
Average.....			9.6	44.5	0.81	
0.5 Sure's salts + 0.28 sodium chloride for 60 days; then 4 Osborne-Mendel salts for 30 days	90	C 4763	13.1	61.1	1.57	Protrusion of one eye
	90	C 4765	12.9	58.9	1.43	Protrusion of eyes; bloody rings
	90	C 4769	13.1	62.5	1.66	
	90	C 4771	12.3	61.1	1.57	Bloody ring around one eye
Average.....			12.9	60.9	1.56	
0.5 McCollum's salts for 60 days; then 1 calcium carbonate added for 30 days	90	C 4799	14.3	61.6	1.60	Depilation
	90	C 4804	12.6	58.6	1.42	Protrusion of eyes
	90	C 4807	12.6	58.6	1.41	Protrusion of eyes
	90	C 4809	12.4	57.3	1.34	Protrusion of eyes
Average.....			13.0	59.0	1.44	
0.5 Sure's salts + 0.28 sodium chloride for 60 days; then 4 Osborne-Mendel salts for 60 days	120	C 4778	14.0	64.7	1.83	Slight protrusion of eyes; deplilation
	120	C 4780	14.0	63.9	1.77	Slight protrusion of eyes
	120	C 4782	11.9	63.6	1.75	Malfunction of hind leg; deplilation
	120	C 4784	....	64.2	1.35	Protrusion of eyes; cataract
Average.....			13.3	64.1	1.68	
0.5 McCollum's salts for 60 days; then 1 calcium carbonate added for 60 days	120	C 4814	13.4	63.3	1.72	Protrusion of eyes; nervous- ness
	120	C 4817	10.9	63.0	1.72	Bloody ring around one eye
	120	C 4819	15.0	63.6	1.75	
	120	C 4822	12.6	64.7	1.83	Slight protrusion of eye
Average.....			13.0	63.6	1.76	
4 Osborne-Mendel salts	90	C 4864	14.0	66.3	1.97	
	90	C 4867	15.4	66.7	2.00	
	90	C 4874	15.4	65.7	1.91	
	90	C 4876	15.0	65.2	1.87	
	90	C 4878	14.0	67.1	2.04	
Average.....			14.8	66.2	1.96	
0.5 McCollum's salts + 1 calcium carbonate	90	C 4880	14.0	65.5	1.89	
	90	C 4882	13.7	64.7	1.83	
	90	C 4884	13.7	65.6	1.90	
	90	C 4886	14.3	65.3	1.88	
	90	C 4888	12.6	64.8	1.84	
Average.....			13.7	65.2	1.87	

*Effects of Diets of Varied Inorganic Salt Content on the Blood and Bones and Also on the General Appearance of Rats—Continued*

Salts in Diet, per Cent	Duration of Feeding, Days	Rat Number	Hemo- globin in 100 Cc. Blood, Gm.	Ash of Dry Fat-Free Bones, per Cent	A/R	Gross Abnormalities During First 60 Days of Experiment
4 Osborne-Mendel salts	120	C 4865	15.0	67.5	2.08	
	120	C 4866	16.1	67.5	2.08	
	120	C 4875	14.0	66.1	1.95	
	120	C 4877	14.6	66.0	1.94	
Average.....			14.9	66.8	2.01	
0.5 McCollum's salts + 1 calcium carbonate	120	C 4879	14.0	66.5	1.98	
	120	C 4881	13.1	66.4	1.96	
	120	C 4883	14.0	66.8	2.02	
	120	C 4885	16.1	66.3	1.97	
	120	C 4889	13.7	66.8	2.02	
Average.....			14.2	66.6	1.99	

deposition of ash in the bones, particularly after sixty days of realimentation. The ash of the bones of such rats was in no case as high as that of the control rats of the same age.

The ratio of the inorganic matter to the organic matter in the bones (A/R ratio) was less than unity in the cases of rats that had been fed on diets low in salt for sixty days. The rise in the inorganic residues of the bones which resulted from the feeding of adequate salt mixtures or of increased amounts of calcium carbonate was, as would be expected, reflected in a rise of the A/R ratios.

The x-ray pictures of the animals that had been fed diets low in salt for thirty days revealed a moderate osteoporosis of the larger bones. The same animals, after sixty days on the same regimen, showed marked generalized osteoporosis as compared with normal animals of the same age. The animals showed a definite increase of calcification in the bony structure after realimentation for thirty days on diets containing 4 per cent of Osborne-Mendel salts or adequate amounts of calcium. The x-ray pictures indicated a greater deposition of inorganic salts in the bones of the rats after realimentation on a diet to which calcium carbonate had been added than in the bones of those animals after realimentation on the ration which contained 4 per cent of Osborne-Mendel salts. According to the chemical analyses, however, almost exactly the same degree of deposition of mineral matter was found in the bones in both groups. The x-ray pictures of the control animals revealed nothing abnormal as far as the bony structure was concerned.

The calcification of the malshapen skeleton, which occurred when adequate amounts of inorganic matter were fed, resulted in an animal with bowed legs and a curved spine. Except for these skeletal defects, after realimentation the animals looked fat, healthy and normal in every way.

#### SUMMARY

Rats deprived of an adequate dietary source of inorganic salts developed certain skeletal abnormalities which in some cases were accompanied by protrusion of the eyes. Histologic examination of the tissues of the eye, the suprarenals and the pituitary gland showed no striking abnormalities; apparent changes in the thyroid gland are being further investigated. A marked osteoporosis of the bones was revealed by x-ray photographs and by chemical examination of the femurs.

The realimentation of such animals with a diet that contained an adequate supply of inorganic salts or a sufficiently high level of calcium resulted in marked calcification of the bones, an increase in the hemoglobin content of the blood and a general improvement in the appearance of the animals.

# UREA CLEARANCE AFTER UNILATERAL NEPHRECTOMY IN DOGS

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By the so-called urea tolerance test, Karsner, Strauss, Moore and Hanzal<sup>1</sup> showed that in the rabbit the removal of one kidney is followed by decreased renal function as regards elimination of urea, and that following the period of decreased capacity the remaining kidney has as great a capacity for elimination of urea as did the two kidneys. With the presentation of the urea clearance test by Möller, McIntosh and Van Slyke,<sup>2</sup> it was evident that the problem could be studied with greater precision and in another animal. The urea clearance in the normal dog was determined by Summerville, Hanzal and Goldblatt<sup>3</sup> and served as a basis for the examination of the results of unilateral nephrectomy in the dog. In this study, their methods as to care, diet and conduct of the urea clearance tests were employed. During the preparation of this paper the report by Ellis and Weiss<sup>4</sup> on urea clearance in man after unilateral nephrectomy appeared, but it was thought useful to place our studies on record even though the findings are essentially the same.

Mature mongrel female dogs of unknown age were used. The body weights were from 9 to 15 Kg. With aseptic precautions, under morphine and ether anesthesia, the right kidney was removed by the extra-peritoneal lumbar route. Each kidney removed was studied grossly and microscopically and was demonstrated to be free from disease. At the termination of the experiments the remaining kidneys were examined, with similar results except for an increase in weight. This increase was from 42 to 48, 46 to 48, 22 to 28 and 19 to 30 Gm. respectively in animals 1, 2, 3 and 4.

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From the Institute of Pathology, Western Reserve University.

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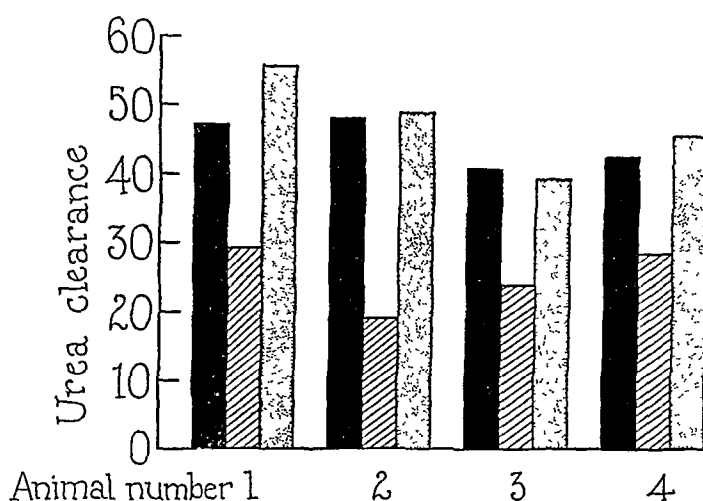
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TABLE 1.—Data on Dog 1\*

Date	Weight, Kg.	Ideal Surface Area for Length, Sq.Mm.	Volume of Urine per Minute, Cc.	Urea in Urine, Mg. per 100 Cc.	Urea in Blood, Mg. per 100 Cc.	Clearance Corrected for Surface Area, Cc.	Comment
5/23/31	12.7	0.5879	1.53	426	17.5	62.8	180 cc. water
			0.41	850	17.5	34.4	
			0.16	1,820	17.3	46.4	
			0.14	1,390	17.3	38.6	
5/24/31	12.7	0.5879	1.60	635	28.4	60.8	317 cc. water
			0.56	1,510	28.4	50.6	
			0.20	2,800	26.8	51.3	
			0.11	3,310	26.8	45.5	
			0.13	3,050	24.0	46.2	
			0.19	3,110	24.0	61.8	
5/25/31	12.7	0.5879	1.63	250	13.7	59.7	317 cc. water
			2.23	205	13.7	69.5	
			2.92	190	13.4	70.5	
			1.38	430	13.4	73.4	
			2.47	224	12.5	75.4	
			1.97	227	12.5	59.7	
6, 2/31	13.8	0.6091	2.18	1,830	111.5	59.9	345 cc. water; 13.8 Gm. urea
			1.10	3,140	111.5	50.7	
			0.75	3,900	101.0	47.6	
			0.41	4,520	101.0	30.7	
6/11/31	14.0	0.6128	0.09	3,260	13.2	78.4	Right kidney re- moved 6 10 31
			0.05	3,260	13.2	69.0	
6/12/31	14.0	0.6128	2.76	294	11.35	116.8	350 cc. water
			0.68	517	11.35	50.2	
			0.49	740	10.4	56.8	
			0.10	2,500	10.4	79.3	
6/13/31	14.2	0.6165	3.37	210	19.4	59.2	335 cc. water
			0.43	890	19.4	31.9	
6/14/31	14.2	0.6165	1.60	1,370	110.8	32.1	355 cc. water; 14.2 Gm. urea
			0.70	2,280	110.8	23.3	
			0.59	2,500	99.6	22.1	
			0.60	2,350	99.6	23.0	
			0.38	2,565	76.4	21.8	
			0.28	3,400	76.4	24.8	
6/23/31	13.6	0.6053	3.23	210	12.5	59.6	410 cc. water
			0.38	640	12.5	33.8	
			1.43	221	12.4	41.2	
7/20/31	9.2	0.5127	0.43	440	8.6	42.9	275 cc. water
			0.32	525	8.6	35.1	
			0.08	1,350	8.0	61.1	350 cc. water
			0.90	185	7.6	42.7	
7/22/31	9.2	0.5127	1.58	95	7.6	38.6	230 cc. water; 9.2 Gm. urea
			1.50	1,480	88.3	42.5	
			0.60	2,215	88.3	29.5	
			0.48	2,460	73.2	31.8	
			0.23	3,610	73.2	29.8	
			0.35	2,580	55.9	34.9	
			0.20	3,705	55.9	37.5	
12/15/31	17.2	0.6673	0.73	1,205	21.6	61.1	430 cc. water: preg- nant and gave birth to 13 pups 12 20/31
			1.30	359	21.6	32.4	
			0.78	732	21.0	40.8	
			0.80	1,315	21.0	33.3	
1/ 7/32	13.6	0.6053	0.18	5,180	38.5	60.4	Starved only a few hours
			0.16	5,570	38.5	62.0	
1/12/32	13.6	0.6053	4.18	1,232	143.4	59.4	340 cc. water; 13.6 Gm. urea
			1.62	3,290	143.4	61.4	
			1.12	3,585	110.3	60.2	
			0.78	4,730	110.3	51.7	
			0.48	5,615	83.3	53.6	
			0.28	6,810	83.3	46.4	
1/14/32	13.4	0.6015	0.26	5,230	47.6	60.8	
			0.23	5,370	47.6	58.4	

\* Only the necessary data are given. All the additional figures employed in determinations of the urea clearance,<sup>2</sup> such as  $\sqrt{V}$ ,  $C_m$  and  $C_c$ , can be calculated from the material given.

The tests recorded as "plus urea" were made after the oral administration of 1 Gm. of urea in 25 cc. of water per kilogram of body weight. Control clearance values with and without urea were obtained for each dog before operation. In order to secure large volumes of urine, water was administered in some cases thirty minutes before the start of a test. Postoperative clearance values with and without added urea were obtained after one, two and three days, one, two and six weeks and six and seven months. In order to conserve space, the complete data for dog 1 only are given in table 1. In table 2 and in the chart the summarized data for the four animals are given. The clearance values have been corrected for body surface in order to compare them with the normal



Average urea clearance, corrected for surface area, in the control (solid black), postoperative (diagonal lines) and recovery (dots) periods following the administration of urea.

TABLE 2.—Average Urea Clearance in the Control, Postoperative and Recovery Periods

Period of Observation	Dog 1		Dog 2		Dog 3		Dog 4	
	— Urea, C/SA*	+ Urea, C/SA	— Urea, C/SA	+ Urea, C/SA	— Urea, C/SA	+ Urea, C/SA	— Urea, C/SA	+ Urea, C/SA
Control.....	56.2	46.9	41.1	48.0	52.1	41.6	58.8	42.7
Postoperative.....	57.9	29.4	37.7	19.1	37.1	23.8	35.1	28.3
Recovery.....	51.2	55.4	51.4	48.8	70.9	39.9	43.7	45.7

\* C/SA = corrected for surface area.

values of Summerville, Hanzal and Goldblatt.<sup>3</sup> The advantages of this corrected value are given in their paper.

From table 2 it is evident that without the added load of urea the clearance was somewhat reduced in only two of the animals in the early postoperative period. With the oral administration of urea, however, the drop in clearance values, namely to 62.7, 39.8, 67.2 and 66.3 per cent

of the original values, is evidently significant. After recovery, the clearance values without and with the administration of urea are well within the normal range. Thus the average values for the four animals at this time are respectively 118.1, 101.6, 96 and 107 per cent of the preoperative figures.

#### CONCLUSION

With the urea clearance test as a criterion, unilateral nephrectomy is followed by a transient period during which the remaining kidney is physiologically deficient. After six months the remaining kidney functions as well as did both kidneys originally. Thus the enlargement of the remaining kidney of the dog is a true hypertrophy in the critical sense of the word. This result agrees with those of previous experiments on the rabbit by the urea tolerance test and with the results of Ellis and Weiss<sup>4</sup> in man with the urea clearance test.



# AMYLOIDOSIS

## EXPERIMENTAL STUDIES

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## I. PRODUCTION OF AMYLOIDOSIS IN MICE

Recent work indicates that amyloidosis is not a degenerative disease but the result of a long-continued metabolic disorder, probably protein in nature. The valuable contributions of Kuczynski,<sup>1</sup> Smetana<sup>2</sup> and Jaffé<sup>3</sup> in the study and understanding of the pathologic process enable one to investigate this condition more adequately.

### METHOD OF STUDY

Four groups of forty albino mice, one group of sixty mice and one group of thirty and one of twenty-five white mice were used. Each group was kept in separate wooden cages with false bottoms to permit the excreta to fall to the lower compartment of the cage.

The diet (stock diet) consisted of powdered whole milk and ground white bread, in proportions of 3 to 2, respectively. In addition, tap water was given.

Amyloid was produced in the manner already described by Smetana, Jaffé and ourselves,<sup>4</sup> consisting, briefly, in daily injections of from 0.2 to 0.4 cc. of a 5 per cent aqueous suspension of sodium caseinate for five successive days each week. The animals to be killed were given injections of from 0.5 to 1 cc. of 10 per cent aqueous solution of congo red in the right lateral tail vein. The spleen, liver and kidneys were sectioned as described.<sup>4</sup> Cultures were made of the sites of the injections of sodium caseinate<sup>5</sup> immediately post mortem, and pieces of the muscle

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From the Pediatric Research Laboratory of the Jewish Hospital and the Pathological Laboratory of the Beth-El Hospital.

1. Kuczynski, M. H.: *Virchows Arch. f. path. Anat.* **239**:225, 1922; *Klin. Wchnschr.* **2**:727, 1923.

2. Smetana, H.: *Bull. Johns Hopkins Hosp.* **37**:383, 1925.

3. Jaffé, R. H.: *Arch. Path.* **1**:26, 1926.

4. Grayzel, H. G.; Jacobi, M.; Maslow, H., and Warshall, H. B.: *Proc. Soc. Exper. Biol. & Med.* **28**:172, 1930.

5. Ushino: *Verhandl. d. deutsch. path. Gesellsch.* **20**:304, 1925.

were taken for histologic study. All the cultures were uniformly sterile. Sections of the muscle showed varying amounts of hyaline degeneration, fragmentation and diffuse polymorphonuclear leukocytic and lymphocytic infiltration, but no abscesses, micro-organisms or local deposits of amyloid.<sup>3</sup>

It has been shown—and on this point the literature has been reviewed extensively and in detail by Wickmann<sup>6</sup> and Schmidt<sup>7</sup>—that the microchemical staining reactions for amyloid vary with its age. Smetana<sup>2</sup> applied congo red, first suggested by Bennhold<sup>8</sup> as a test for amyloid *intra vitam*, to its pathologic study. He and others showed that this dye reveals the presence of amyloid before any other dye. This dye has therefore been used in our experiments except when noted. Application of the dye to surgical and autopsy specimens showing hyaline and granular degeneration produced none of the tinctorial pictures obtained with amyloid. When stained with congo red amyloid retains its color for about six months, slowly fading thereafter; the morphology is not affected by the loss of color. Sections must not be allowed to remain in any of the dehydrating alcohols, particularly 95 per cent, for more than from one-half to one hour, lest the red or pink-red color be removed or so weakened as to assume a purplish hue.

Until 10 or 15 injections of sodium caseinate had been given, there were no demonstrable gross or microscopic alterations. After 20 injections, the enlarged spleen was soft and more bloody and showed more sharply outlined follicles than usual. Microscopically, the sinuses, particularly the perifollicular sinuses, were tremendously distended with erythrocytes; there were no evidences of phagocytosis. Numerous giant cells appeared in the sinuses and in the red and white pulp. They were discrete, round or roughly pentagonal and measured from 15 to 25 microns. Their cytoplasm was unstained and finely granular. They contained from three to five or six well defined, round nuclei of the vesicular type, generally grouped at one pole of the cell with small amounts of finely granular chromatin scattered irregularly on a fine linin network. No cell inclusions or vacuoles were demonstrable. These cells appeared most numerous in the immediate perifollicular sinuses and red pulp. The reticular cells of this region seemed more distinct and were swollen; the cytoplasm was unstained and homogeneous. The littoral cells presented a similar appearance; the nucleus still lay near the inner border. Moderate numbers of immature polymorphonuclear leukocytes were present between the reticular cells. The lymphoid cells of the red and white pulp seemed fewer and, in the white pulp, compressed. The malpighian corpuscles were sharply outlined as small, dense masses of lymphocytes with but a faint suggestion of secondary follicles. The trabeculae and arteries showed no demonstrable changes. These changes will be referred to as precursory amyloid changes in subsequent sections.

Essentially the same appearances were present after 23, 26 and 29 injections. After the thirty-second injection the macrophages showed minute pink to pink-red crystalline or granular areas in the cytoplasm. These appeared in the central zones of the macrophages, both those within the sinuses and those in the pulp stroma. The other structures were similar to those described after 29 injections.

After 37 injections the spleen appeared smaller and less bloody. Microscopically, the macrophages contained large amounts of pink-red inclusions, which occupied the greater portion of the cell, with a narrow clear zone between these masses and the periphery of the cell, the masses appearing to lie within an intracellular

6. Wickmann, G.: Beitr. z. path. Anat. u. z. allg. Path. **13**:487, 1893.

7. Schmidt, M. B.: Verhandl. d. deutsch. path. Gesellsch. **7**:2, 1904.

8. Bennhold, H.: Klin. Wchnschr. **3**:1711, 1924.

vacuole. Such macrophages were more numerous and were arranged in linear manner, partially filling the sinuses surrounding the follicles. The reticular cells also contained similarly tinted crystalline inclusions, as did the littoral cells. The groundwork around the reticular fibers appeared thickened, those in the perifollicular zone showing pale pink-red tinting occupying short segments. The fibers themselves were unaltered. The remaining cellular, vascular and trabecular elements appeared as in the immediately preceding group.

After 50 injections the groundwork around the reticular fibers was thicker and more deeply tinted, and formed a latticework, which was most pronounced around the follicles, being gradually lost in the pulp, where small similar appearances were noted. The walls of the sinuses were outlined by a thickened, perisinus, pink-red tinted, perifibrillary groundwork, which could not be distinguished from the increased, swollen littoral cells the cytoplasm of which was filled entirely with pink-red inclusions and the nucleus of which was pyknotic. The reticular cells presented a similar appearance. These cells lay adjacent to or overlying the thickened, tinted, reticular fiber groundwork. Macrophages were numerous but fewer than in the previous sections; the outlines were indistinct, and the cytoplasm seemed to taper off into fine filaments which crisscrossed across the lumen of the sinus and were lost in the adjacent pulp. They were grouped and appeared fused in places, particularly in the perifollicular red pulp. Those within the sinuses had taken a position adjacent to the wall of the sinus, from which they could not clearly be distinguished in places. Their cytoplasm, like that of the littoral cells, was tinted uniformly a deep pink or pink-red, with a few peripheral crystalline areas. The nuclei were generally fused and had indistinct outlines and a pyknotic appearance. The sinuses were still patent but contained far fewer erythrocytes than in the preceding sections, and appeared narrowed. The follicles were small and irregular. In the outer wall of an occasional arteriole a few small pink-red crystalline granules appeared. These, however, frequently appeared within cells resembling reticular cells, so that it was impossible to say whether or not they were within the arteriolar wall.

After between 55 and 65 injections the spleen was still smaller, dry and lusterless on cut surface and without grossly visible follicles. The cut surface, in thin sections, appeared as an interlacing coarse lattice of pink-red strands of varying width between which minute pinpoint-sized nodules could be distinguished indefinitely. Microscopically, the red and white splenic pulp was composed of huge masses of homogeneous deep red substance in which appeared, irregularly, isolated lymphocytes, a few immature leukocytes and a few distorted, dense masses of lymphocytes which suggested lymphoid follicles but showed no definable structure. Numerous spaces, either narrow and slitlike or somewhat dilated, containing erythrocytes were seen in the red masses, lined only infrequently by flattened endothelial cells. Numerous macrophages, generally in agminate groups and with faint cell outlines, were seen in these masses. They blended indefinitely with the surrounding masses; their nuclei were pyknotic and showed numerous bizarre forms. The arterioles were surrounded by homogeneous pink-red masses continuous with the remainder of the red masses of the sections. Their endothelial cells were hyperplastic, and their lumens were narrowed. The dense red masses were penetrated by prominent trabeculae.

In these sections and in the immediately preceding one both the metachromasia with methyl violet and the pink when treated with iodine were obtained, the latter color changing to gray-blue or pale green-blue on the addition of sulphuric acid, as described also by Jaffé.<sup>3</sup> In the sections of animals receiving more than

45 injections, the metachromasia with methyl violet was shown by the intracellular and fibrillar pink-red areas tinted by vital staining. The red tinting obtained on the addition of iodine and its subsequent tinctorial changes on the addition of sulphuric acid were evidenced only faintly by the larger intracellular inclusions in the macrophages, which appeared crystalline.

The perifibrillary groundwork reticulum of the pulp and the perisinus wall and the littoral and reticular cells, which were red-tinted with the vital staining, were not tinted by the iodine. After less than about 45 injections no tinctorial changes were observed with iodine and only doubtful metachromasia with methyl violet was given by an occasional macrophage. The reticular fibers became fragmented and finally disappeared as the surrounding groundwork increased in density and depth of color.

*Liver.*—Like the liver of other small laboratory animals (rats, guinea-pigs and dogs), the liver of mice showed areas of cellular infiltration the significance of which is unsettled. Unlike these animals, however, these areas are comparatively infrequent in mice, are generally located around vessels in the portal field, are not sharply defined and are composed of lymphocytes and large mononuclears.<sup>9</sup> The Kupffer cells, while visible, are never prominent in sections from normal animals. No protoplasm is apparent as a rule, nor, normally, is there any evidence of phagocytosis.<sup>10</sup>

No structural changes were apparent grossly in the livers of the mice given injections throughout the experiment, nor were any demonstrable histologically until 20 injections had been given. Thereafter, until the thirtieth injection, two changes became manifest progressively in an almost quantitatively increasing manner. The Kupffer cells, previously inconspicuous and visible only as nuclei, increased in number and size, so that at the twenty-seventh injection they appeared as closely studded, rounded cells projecting into the sinusoidal lumen but still contiguous with the liver cords. Their cytoplasm was easily visible, unstained and finely granular; the nucleus, still densely basic, appeared as a well demarcated, round, eccentrically placed structure. These cells appeared most numerous in the central and midzonal portions of the lobules. Surrounding nearly every central vessel there appeared a clear, narrow, slitlike space in which were polymorphonuclear leukocytes of adult form and a few small mononuclear cells. These cellular groups—few and arranged in linear manner at the twentieth injection—increased in number, so that at the thirtieth injection they appeared as wide collars of densely packed leukocytes around the endothelium of the central vessels compressing the surrounding parenchymal cords. The endothelial cells of the central vessels at this stage appeared swollen. The sinusoids of the pericentral third of the lobules contained moderate numbers of polymorphonuclear leukocytes and an occasional small mononuclear cell, the cellular content becoming less marked peripherad. These appearances will be referred to as precursory amyloid changes in subsequent sections.

After the thirtieth injection, the cellular infiltrative changes remained unchanged until the fiftieth injection, when, as the amyloid deposits increased, the cell masses slowly and irregularly became smaller, the cells fragmented so that after 65 injections the perivascular infiltration appeared as nuclear fragments in the homogeneous perivascular amyloid, while in the adjacent sinusoids a few leukocytes were seen between the enormously enlarged and numerous Kupffer cells. At no

9. Jaffé, Rudolf: *Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere*, Berlin, Julius Springer, 1931, p. 233.

10. Jaffé, R. H.: *Arch. Path.* 2:149, 1926; footnote 3.

stage was there any evidence of migration of any of the wandering cells through the endothelial lining of the central vessels.

After 28 injections, some of the Kupffer cells showed small crystalline areas in the cytoplasm, radially arranged and irregularly located in the cell, with a tendency to appear centrally, either unstained or having a slight brown-pink color. The nucleus was somewhat less homogeneously basic, but *no distinct chromatin* structure was discernible. After 32 injections, these masses had assumed a more definite pink tint. Thereafter the tint became progressively deeper and changed gradually through a pink-red to an orange-red and finally to a deep red (51 injections). Concomitantly, the crystalline masses increased in size to occupy the entire cell (47 injections), the crystalline appearance being still distinct. At this stage the central zone of the crystalline region had lost its crystalline appearance, now appearing as a homogeneous area, more deeply red. After 51 injections the greater portion of the red-tinted mass appeared homogeneous; after 56 injections the cytoplasm of the entire cell appeared homogeneous and deep pink-red.

The nucleus meanwhile had become more vesicular (41 injections) and irregular in outline (50 injections), and finally appeared in fragments (62 injections) scattered at one end of the cell or as a compressed, elliptic, vesicular but heavily chromatic, irregularly outlined body eccentrically displaced toward the liver cord surface. In this stage occasional cells with intact nuclei and with crystalline cytoplasmic inclusions appeared free within the sinusoidal lumen.

Until the fiftieth injection the outline of the Kupffer cells was distinct. Thereafter the cells appeared to fuse, so that large masses of either homogeneous red or, more infrequently, crystalline pink-red substance appeared surrounded by several flattened nuclei. These masses lay in close apposition to the cords, compressed and caused some atrophy of the cords and partially occluded the sinuses. After the sixty-fifth injection homogeneous masses of this material, in which a few nuclear fragments were scattered, completely filled many sinusoids; the atrophy of the cord in these regions was pronounced. At no time did we observe any cellular lining separating the crystalline or homogeneous tinted masses from the lumen of the sinus, nor did the nodular and linear thickenings of the capillary walls, extracellularly, appear in our sections (as reported by Jaffé<sup>3</sup>). The fat droplet inclusion within the Kupffer cells and the giant cell formation of the type described by Jaffé were never apparent.

After the thirtieth injection the endothelial cells lining the central vessels appeared swollen; the surrounding tissue was infiltrated with cells as described. The endothelial basement membrane seemed thickened but not pigmented or with overlying crystalline deposits. After 35 injections there appeared among these cells a few large mononuclear cells similar to those described in the spleen. After the forty-eighth injection the perivascular infiltrate contained numerous such mononuclears, their cytoplasm containing crystalline pink-red inclusions. After 51 injections there appeared homogeneous pink-red masses in this space. The endothelial basement membrane was definitely thickened and had assumed a red tint. There were, however, no crystalline masses except those within the now numerous, and larger mononuclear cells, some of which appeared fused.

The extracellular homogeneous masses quickly increased in width, the discontinuous globules fusing rapidly so that in sections after the sixty-fifth injection most of the central vessels were surrounded by a zone, of varying width, of homogeneous, deep pink-red, glassy-looking substance in which were scattered nuclear fragments, a few faintly outlined mononuclears and polymorphonuclear leukocytes and a very occasional small, faintly crystalline, pink-brown mass. Near the latter one or more flattened and generally fragmented nuclei were present; no cell outlines were, however, apparent. The basement membranes could no longer

be distinguished, the red collar extending from the surrounding atrophic hepatic cords to the swollen endothelial cells, the bases of which seemed lost in the pink-red zone. No tinting or crystalline, globular, vacuolar or homogeneous inclusions could be seen in the venous lining cells.

Except for the compression and atrophic changes described in the hepatic cords, no relevant alterations were apparent in the liver cells. Even after 70 injections, we could observe no changes in the arterial walls. The cellular infiltration described by Jaffé, Maximow<sup>10a</sup> and others around the portal vessels showed no alterations in size, density or cellular character.

*Kidney.*—The kidney becomes involved relatively late. In our animals no changes were demonstrable with any regularity until 35 injections had been given,

TABLE 1.—*Degree of Amyloidosis in Albino Mice Following the Stated Number of Injections\**

Number of Injections	Number of Mice in Group						
	25	30	40	40	40	40	60
10.....	..	..	..	..	..	0	0
17.....	..	..	..	..	..	0	0
23.....	0	..	..	..	..	0	0
24.....	..	..	..	..	0	..	..
29.....	0	+	..	..	..	+	0
30.....	..	..	..	..	0	..	..
32.....	+	..	+	0	..	..	..
35.....	..	+	..	..	++	+	+
36.....	..	..	..	+	..	..	..
42.....	++	..	+	++	+	..	++
45.....	..	++	..	..	..	..	..
47.....	..	..	++	..	..	++	..
48.....	..	..	..	..	..	..	++
50.....	..	..	..	..	+++	..	..
51.....	+++	++	..	..	..	..	..
56.....	..	..	++++	+++	..	..	+++
57.....	..	..	..	..	+++	+++	..
62.....	..	..	+++	++	..	..	+++
65.....	..	..	..	..	++++	..	..
66.....	..	..	..	+++	..	+++	..
75.....	..	..	..	..	++++	..	..
78.....	..	..	..	++++	..	++++	..
89.....	..	..	++++	..	..	..	+++
105.....	..	..	..	+++	..	..	..

\* 0 indicates no amyloidosis; +, early amyloidosis; ++, moderate amyloidosis; +++, moderately advanced amyloidosis; +++++, advanced amyloidosis.

when all the mice showed a moderate hyperplasia of the glomerular endothelial cells, with consequent narrowing or total occlusion of the capillary loops. In a few glomeruli these cells had lost distinct outlines, appearing as a cytoplasmic mass containing varying numbers of nuclei, their position in the glomerulus showing no regularity. No thickening of the basement membrane or tinctorial changes were apparent, except for the appearance in the renal sections of one mouse of small pink-red crystalline areas (as described in sections of the spleen and liver) in the cytoplasmic zones of the fused, giant cell-like, intraglomerular areas. In the interstitial tissue surrounding such glomeruli were small foci of lymphocytes and a few polymorphonuclear leukocytes. There were no apparent changes in the tubules, vessels or interstitial tissue.

Thereafter only the glomerular endothelial hyperplasia and the cellular infiltration of the interstitial tissue were apparent in sections obtained after 38, 42, 48 and

<sup>10a</sup> Maximow, A. A.: Textbook of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 392.

51 injections. After 56 injections had been given, small crystalline areas were seen in glomeruli presenting the appearance of the unusual example noted after the thirty-fifth injection. In these sections a few giant cells in the interstitial tissue contained minute crystalline inclusions of a similar character, while here and there a few small, homogeneous, pink-red strands appeared among the leukocytic masses in the interstitium. These were chiefly in regions showing the macrophages.

After 65 injections—at which point this experiment terminated—there were slightly more numerous extracellular deposits, and the intraglomerular deposits were larger and surrounded by nuclei—pyknotic or even fragmented. At no stage was there any evidence of change in the epithelial cells or of vascular alteration.

*Rats.*—Following the technic described, seven rats were given 138 injections of sodium caseinate. They showed none of the clinical appearances associated with visceral amyloidosis. There was no evidence of any of the changes described in the organs of our mice. The frequent focal necrosis and leukocytic infiltration of the liver and the widespread diffuse and focal polymorphonuclear leukocytic content in the spleen have been described as of spontaneous occurrence. They may be an expression of spontaneous infection with *Bartonella muris*.<sup>9</sup>

#### COMMENT

Amyloidosis was produced in albino mice by the subcutaneous or intramuscular injection of a 5 per cent aqueous suspension of sodium caseinate. Invariably, definite evidence of amyloidosis was obtained with from 30 to 32 inoculations and became more striking and more marked with successive injections.

In the earliest stages, the amyloid appeared as radially arranged crystalline masses in the wandering macrophages and in the phagocytic fixed tissue cells, such as the littoral and reticular cells of the spleen and the Kupffer cells of the liver. After a considerably later period, similar crystals were found in the swollen, proliferated and fused glomerular endothelial cells of the kidney. As the number of injections was increased, the intracellular amyloid increased in amount. It assumed a deeper red tint. It progressively lost its crystalline character, changing to a homogeneous, glassy appearance. The first evidence of this appeared in the central portions of the deposits. Concomitantly, necrobiotic nuclear changes appeared in the cells. The cells fused. The amyloid finally appeared as homogeneous masses, showing no cellular structure. These masses became continuous with the extracellular masses that had meanwhile developed.

The final picture was one in which, depending on the number of injections, the visceral parenchyma was markedly compressed and atrophic between the masses, or was wholly replaced by the amyloid. The process made its first appearance in the spleen. Following this, the liver was involved, and then the kidney (only to a far lesser extent and after a considerable lapse of time). The process progressed at a

much more rapid rate in the spleen than in the other organs. The suprarenal glands, the intestines and the other parenchymatous organs were involved in the process.<sup>3</sup>

Amyloidosis can be produced in certain animals by the parenteral introduction of a variety of substances. Although in most instances the inoculating material was of protein composition, yet in some cases it was non-nitrogenous, as, for example, silicates and selenium. However, whole protein compounds must be used, and not their intermediary or split products. With the use of the latter, amyloidosis will not occur.<sup>11</sup>

Furthermore, it was the number of injections and the duration of the administration of the material, and not the quantity introduced at each injection, that influenced the pathologic process. This was pointed out by Jaffé<sup>3</sup> and confirmed by us. Whether 0.2 or 1 cc. of the solution was used, the rate of production of this condition was the same.

It must be added that, as will be shown in parts III and IV, diet exercises an influence on the production of amyloidosis. A thoroughly adequate diet and one containing an abundance of vitamins A and B will retard but will not prevent the development of this process. With different diets, the same animals will vary in their "time-response" to the formation of amyloid. Consequently, for comparative studies, the importance of similarity of the intake of food is obvious and has not been stressed at all.

Although there was a variability in the response of animals to the production and development of amyloidosis, yet, in our experience, all albino mice eventually acquired this disease. However, there are several species of animals in which, to date, and with present methods of production of amyloidosis, this condition cannot be produced. This difficulty was encountered by others.<sup>12</sup>

The mechanism in the production of amyloidosis and the origin of amyloid are as yet unknown. Several explanations have been offered. By gathering all the known data, one can construct a plausible theory which to date offers a satisfactory explanation.<sup>13</sup>

The injected material can be dismissed as the possible source of the amyloid, or even as one of the contributors. In the first place, the amount introduced does not influence the rate of production of amy-

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11. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 469.

12. (a) Lucke, B., and Markley, L. A.: *Proc. Soc. Exper. Biol. & Med.* **25**:642, 1928. (b) Robinson, B. L., and Thatcher, H. S.: *ibid.* **27**:580, 1930.

13. (a) Neuberg: *Verhandl. d. deutsch. path. Gesellsch.* **7**:19, 1904. (b) Davidson, C.: *Virchows Arch. f. path. Anat.* **150**:16, 1897; **155**:382, 1899; **192**:226, 1908. (c) Dietl, K.: *Beitr. z. Klin. d. Tuberk.* **51**:18, 1922. (d) Letterer, E.: *Zentralbl. f. inn. Med.* **47**:417, 1926. (e) Kuczynski.<sup>1</sup> (f) Smetana.<sup>2</sup>



loidosis. It is wholly dependent on the number of inoculations and the period of time during which the irritant is administered. In the second place, the composition of the amyloid is independent of the chemical nature of the substance given parenterally. Different protein compounds and, more striking still, certain nonprotein compounds and inorganic substances will produce amyloidosis and the deposition of practically identical lardaceous material.<sup>14</sup>

That the injected substance causes an internal disorder is not questioned. Its exact nature is unknown. Perhaps it produces a disturbance of protein metabolism.

The introduction of a foreign toxic substance results in the destruction of body protein.<sup>15</sup> It was shown by Mendel and Rockwood<sup>16</sup> that the intravenous administration of edestin or casein to a dog caused the metabolism of protein which was far in excess of that ordinarily occurring in the dog plus that of the introduced protein.

It has been definitely proved by chemical analysis that amyloid is a complex protein substance.<sup>14</sup> Its mother substance must be of protein composition. This might be supplied from ingested protein, from "deposit protein" or from tissue protein. That an excessive and almost exclusive intake of protein might cause amyloidosis must still be considered possible in view of Kuczynski's work.<sup>1</sup> He, and later Smetana,<sup>2</sup> produced amyloidosis in albino mice by the feeding of a rich protein diet of cheese, bread, protein from chicken eggs and milk. However, it is doubtful if it can be produced so readily, if at all, in other animals.

It has been demonstrated by Voit and Landois<sup>17</sup> that ingested protein and deposit protein are readily metabolized, but that living tissue protein is quite resistant. This indicates that the organism is far better able to metabolize and dispose of the products of exogenous than of endogenous protein metabolism. Consequently, excessive destruction of tissue protein with liberation of such protein would place a severe metabolic burden on the animal.

That protein can circulate in the blood and be transported from one tissue to another is proved by the work of Miescher.<sup>18</sup> He showed that the marked development of the genital organs of starving salmon occurred as a result of the transportation in the blood of protein from the muscles.

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14. (a) Kravkoff, N. P.: *Arch. f. exper. Path. u. Pharmakol.* **40**:196, 1897. (b) Hanssen, O.: *Biochem. Ztschr.* **13**:185, 1908. (c) Moyeda, M.: *Ztschr. f. physiol. Chem.* **58**:475, 1909. (d) Eppinger, H.: *Biochem. Ztschr.* **127**:101, 1921.

15. Lusk, G.: *The Science of Nutrition*, ed. 4, Philadelphia, W. B. Saunders Company, 1928, p. 194.

16. Mendel, L. B., and Rockwood, E. W.: *Am. J. Physiol.* **12**:350, 1904-1905.

17. Quoted by Lusk,<sup>15</sup> pp. 80 and 90.

18. Quoted by Lusk,<sup>15</sup> p. 80.

However, the infiltration of amyloid in certain tissues is more than a simple precipitation or separation of circulating protein. The absence of universal deposition of amyloid in the organism, particularly at the beginning of the disease, and the constancy with which certain sites are elected suggest that some other factor or factors enter into this process. The early localization of amyloid material in the wandering and fixed cells of the reticulo-endothelial system in the spleen and liver shows an interaction which calls for an explanation. The intracellular inclusion of amyloid by the wandering macrophages and in the phagocytic fixed tissue cells of the spleen and liver suggests phagocytosis by these cells of the circulating toxic protein. For a time only intracellular amyloid is evident objectively. This may denote absence or such insignificant amounts of extracellular material that they cannot be demonstrated histochemically. With the continued progression of the process, extracellular amyloid becomes apparent, and soon fuses with the amyloid from the cells, which, about this time, undergo disintegration. The extracellular deposition of amyloid may be due to the exhaustion and inability of the fixed and wandering cells of the reticulo-endothelial system to cope with the increasing demands. Hypertrophy and hyperplasia cannot go on indefinitely. Phagocytosis cannot keep pace with the continued supply of newly liberated toxic protein. Supersaturation of this protein occurs. However, simple precipitation of the material will not explain the failure of generalized deposition of amyloid in all the crevices of the tissue and the occurrence of this deposit about the reticulo-endothelial cells. A more probable explanation may be that the exhaustion, death and disintegration of the phagocytosed cells liberate ferments, enzymes or other chemical substances which are necessary for the precipitation and deposition of the extracellular amyloid. It is probably more than a coincidence that the extracellular amyloid is observed just about the time that the phagocytic cells begin to disintegrate.

This raises the question whether the latter is actually a deposit product or a homogeneous fusion of the disintegrated phagocytic cells which have lost all trace of such cellular character. To date all histologic studies fail to disprove or negate the latter possibility.

## II. RESORPTION OF AMYLOID

The exact chemical composition of amyloid has not been determined. Difference of opinion still exists in regard to its constituents. Kravkoff and others<sup>19</sup> considered its chemical nature that of a conjugated pro-

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19. Kravkoff.<sup>14a</sup> Friedreich, N., and Kekule, A.: *Virchows Arch. f. path. Anat.* **16**:50, 1859. Kühne, W., and Rudneff: *ibid.* **33**:66, 1865. Oddi, R.: *Arch. f. exper. Path. u. Pharmacol.* **33**:377, 1894.

tein, probably chondroitin-sulphuric acid. Hanssen<sup>14b</sup> and Henlein<sup>20</sup> noted an excess of sulphur in the tissues containing the amyloid material. However, when amyloid masses were obtained in purer form, i. e., devoid of the surrounding tissue, and were analyzed, no sulphur was found.<sup>21</sup> In addition, Eppinger<sup>14d</sup> showed that this complex protein substance varied in its composition and consisted of a number of different amino-acids.

The amyloid material in the tissues is an aggregate of complex protein molecules which are not always and permanently of the same composition. With its continued residence in the organs and in the presence of the factor inciting its formation, the amyloid material probably undergoes certain chemical changes and become more stable. The occurrence of this transformation is indicated by the acquisition of additional staining properties<sup>11</sup> and its increasing resistance to digestive ferments and to chemical changes.<sup>22</sup>

In man, once evident clinical signs of amyloidosis are present, the condition usually progresses and terminates fatally. As a rule, the underlying condition for the production of lardaceous changes is present. There are very few reports of cases of the disappearance of amyloidosis and recovery.<sup>22</sup> In all of these instances, complete and radical eradication of the suppurative focus was accomplished before the regressive changes in the amyloid occurred.

One of us (H. G. G.), with the administration of a powdered whole liver preparation, succeeded in obtaining clinical evidence of the disappearance of amyloid in the presence of active tuberculous suppuration of bone. The recession of the markedly enlarged liver and spleen in these cases, with the disappearance of other associated symptoms, was considered presumptive evidence of resorption of amyloid substance. A preliminary report of these results was made by Whitbeck.<sup>23</sup>

Stephanowich and Dantschakow,<sup>24</sup> using living bacterial cultures for the production of amyloidosis, were unable to demonstrate resorption. This, however, may have been due to the continued presence of the infection after the cessation of the injections.

Letterer<sup>13d</sup> did not observe resorption in amyloidosis produced experimentally by the injection of sodium caseinate.

On the other hand, Klebs and Wickmann<sup>24</sup> were of the belief that amyloid could be resorbed, and Kuczynski<sup>1</sup> noted resorption in mice.

20. Heinlein, H.: *Arch. f. exper. Path. u. Pharmacol.* **149**:119, 1930.

21. Eppinger.<sup>14d</sup> Lucke, B.: *Proc. Path. Soc. Philadelphia* **42**:19, 1921.  
Hanssen.<sup>14b</sup>

22. Fox, A. R.: *Brit. M. J.* **1**:571, 1924. Walker, G. F.: *Lancet* **2**:120, 1928.  
Waldenström, H.: *Acta chir. Scandinav.* **63**:479, 1928. Schmidt.<sup>7</sup>

23. Whitbeck, B. H.: *J. Bone & Joint Surg.* **14**:85, 1932.

24. Quoted by Morgenstern.<sup>25</sup>

Morgenstern,<sup>25</sup> in his work with albino mice, concluded that resorption of amyloid occurred spontaneously following the cessation of the injections of sodium caseinate and the lapse of a sufficiently long period.

#### SPONTANEOUS RESORPTION OF AMYLOID

In part III we shall call attention to the retarding influence of powdered whole liver on the production and development of amyloidosis. This, coupled with the apparently favorable effect of the liver preparation on clinical cases of amyloidosis, led us to study more adequately the question of spontaneous resorption of amyloid and the rôle of the liver product on this retrogressive process.

#### METHOD

Two hundred and forty albino mice were placed on a stock diet of powdered whole milk, bread and water. Five successive daily injections of a 5 per cent solution of sodium caseinate were given, and were omitted during the next two days. This procedure was repeated weekly, the dose varying from 0.2 to 0.4 cc.

Six cages, each containing forty mice, were segregated for this study. After 20 injections, two cages were set aside. The mice in these two sets were no longer inoculated. One group was allowed to continue on the stock diet. To the stock diet of the other group, a powdered whole liver preparation, forming 10 per cent of the diet,<sup>26</sup> was added. Similarly, after 29 and 40 injections, respectively, two groups of mice were isolated.

After the cessation of the injections, two or three mice were killed at varying periods. In the case of groups 1 and 2, this method was carried out at the end of thirty, sixty-one, ninety-one, one hundred and nineteen and one hundred and seventy-five days. The mice of groups 3 and 4 were studied after twenty-eight, fifty-eight, eighty-nine, one hundred and nineteen and one hundred and sixty-one days, and the mice from groups 5 and 6 after thirty-one, sixty-three, ninety-one, one hundred and twenty-one and one hundred and forty-seven days.

In this manner we were able to study the question of resorption of amyloid at various stages in the development of the process, and to compare the influence of the diet containing powdered liver with that of the stock diet.

#### INTERPRETATION OF STAGES IN RESULTS

In general, we have designated as precursory changes those appearances in the splenic or hepatic sections which antedate the appearance of the intracellular inclusions and the later extracellular, red-tinted, glassy-looking substance.

Moderate amyloidosis designates the presence of larger intracellular, pink-tinted inclusions in the macrophages of the spleen. The littoral and reticular cells present similar smaller intracytoplasmic inclusions. A few thickened, pink-red, perireticular groundwork fibers may be seen in the pulp generally in regions showing the intrasinus inclusions. The liver shows crystalline, pink-red, intracytoplasmic inclusions in a few enlarged and hyperplastic Kupffer cells, and perhaps a leukocytic collar around the central vein.

25. Morgenstern, Z.: *Virchows Arch. f. path. Anat.* **259**:698, 1926.

26. Osborne, T. B.; Mendel, L. B.: Washington, D. C., Carnegie Institution, 1911, no. 156, pt. 2, p. 86.

Moderately advanced amyloidosis is used to signify in the spleen larger extracellular pink-red masses deeper in color causing compression and later atrophy of the normal pulp and follicular elements. The liver shows more numerous Kupffer cell involvement and crystalline inclusions containing macrophages and extracellular red homogeneous masses among the perivascular leukocytes.

Advanced amyloidosis indicates further advance of the extracellular masses to replace the greater portion of the spleen by homogeneous red masses in which fragmented macrophages, polymorphonuclear leukocytes and lymphocytes and distorted follicles and compressed sinuses are seen. In the liver most of the central vessels are surrounded by a mass of dense, acellular, homogeneous, pink-red substance, and nearly every Kupffer cell contains large amounts of similar material. In very advanced cases small, crystalline, pink-red inclusions may be seen in the cytoplasm of fused and hyperplastic glomerular endothelial cells of the kidney.

TABLE 2.—*Resorption of Amyloid Under the Influence of the Stock Diet and a Stock Diet Containing Powdered Whole Liver\**

Group	Number of Injections	Pathologic Finding at Termination of Injection	Diet During Period of Injection	Diet During Period After Injection	Pathologic Finding at Different Periods After Cessation of Injections						
					1 Mo.	2 Mos.	3 Mos.	4 Mos.	4½ Mos.	5 Mos.	5½ Mos.
1	20	±	Stock diet	Stock diet	+	+	+	+	..	..	±
2	20	±	Stock diet	Stock diet and powdered liver	+	+	+	±	..	..	0
3	30	+	Stock diet	Stock diet	+	+	+	++	++	+++	..
4	30	+	Stock diet	Stock diet and powdered liver	±	0	0	±	0	0	..
5	40	++	Stock diet	Stock diet	++	++	++	++	+++	..	..
6	40	++	Stock diet	Stock diet and powdered liver	+	+	±	±	±	..	..

\* ± indicates precursory amyloid changes; +, early amyloidosis; ++, moderate amyloidosis; +++, advanced amyloidosis.

Control animals killed after 20 injections showed only the precursory splenic and hepatic changes. After 30 and 40 injections this group presented early and moderately advanced amyloid alterations, respectively.

The results of this experiment are shown in table 2.

#### COMMENT

After the cessation of the injections, spontaneous resorption of amyloid in albino mice was noted only in the animals belonging to the group that had received very few inoculations and had shown only the precursory or the earliest evidences of amyloidosis (table 2). Those showing very definite signs of this condition failed to reveal any histologic evidence of retrogression. Furthermore, although the administration of the inciting agent was stopped, once definite amyloidosis set in there occurred apparently an accentuation of this process. After a varying period there was more marked and more extensive microscopic evidence of amyloidosis.

In view of the discontinuance of the injections, the more marked objective evidence of amyloidosis that was noted histologically may be due to one of two possibilities. The mere withdrawal of the external agent that provoked the destruction of tissue protein does not necessarily cause an immediate cessation of the morbid process. The latter, once initiated, may continue for some time and thus increase the amount of amyloid in the tissue. Another explanation may be offered. The material contributing toward the formation of amyloid undergoes chemical change during its residence in the tissue. With successive transformations it becomes more visible or can be more readily demonstrated by the present methods of staining.

On the other hand, after the inoculations were stopped, resorption of amyloid in early and in moderate cases of amyloidosis was observed in albino mice that were given a diet to which a preparation of powdered whole liver had been added. Moderately advanced and advanced cases of amyloidosis failed to show any evidence of retrogression of this process.

As mentioned, the amyloid material undergoes chemical change in the tissue, and apparently becomes increasingly more stable and more resistant to disintegration and decomposition. This probably accounts for the failure of spontaneous resorption except in the earliest stages, and of resorption following the use of the liver product in the animals in which the changes progressed to the stage of moderately advanced amyloidosis.

The favorable influence of the powdered whole liver on resorption may be due to the presence of an active potent principle in the liver, or to its abundant supply of reticulo-endothelial cells with its chemical products. The probable important rôle of the reticulo-endothelial system with its fixed and wandering cells in this pathologic condition was described in part I.

Whatever may be the final explanation, there is a retarding influence of the powdered whole liver on the formation and production of amyloidosis.<sup>12a</sup> Resorption is accelerated by liver once the injections are discontinued, provided that advanced amyloidosis has not set in.

### III. INFLUENCE OF DIETARY INGREDIENTS AND INGESTED CHEMICAL SUBSTANCES ON THE PRODUCTION OF AMYLOIDOSIS

Since the basic nature of this disorder is metabolic, qualitative differences in diet may exercise an influence on the disease. Consequently, the rôle of diet, certain chemical substances, a desiccated whole liver preparation and two specific fractions of the liver was investigated.

Kuczynski<sup>1</sup> was the first to describe the production of amyloidosis by the feeding of a diet consisting almost exclusively of cheese. This

was confirmed by Smetana,<sup>2</sup> but not by others.<sup>27</sup> Jaffé<sup>3</sup> noted the retardation of the production of amyloidosis by the addition of small amounts of cholesterol to the diet. Except for these studies, investigators in this field did not concern themselves with the rôle of diet. In fact, they did not even mention the nature of the food which was given the animals.

#### METHODS AND RESULTS

Thirteen different studies were carried out (table 3). The same procedure was employed as outlined in part I.

1. *Stock Diet*.—The results in these groups of mice are described in part I.

2. *Adequate Diet*.<sup>28</sup>—This group consisted of sixty mice. The diet included: yellow cornmeal, 64 Gm.; linseed meal, 10 Gm.; commercial casein, 6 Gm.; ground alfalfa, 2 Gm.; sodium chloride, 9.5 Gm.; calcium carbonate, 0.5 Gm.; whole powdered milk, 15 Gm., and cod liver oil, 2 Gm.

Pathologic studies were carried out after 33, 42, 47, 56, 62, 80 and 89 injections of sodium caseinate.

No changes were apparent until after 62 injections, at which time the spleen showed early evidences of amyloidosis. The liver and kidneys remained unaltered.

After 81 injections moderate amyloidosis set in. This condition became more marked with an increasing number of inoculations.

3. *Stock Diet Plus Powdered Whole Liver*.<sup>29</sup>—Two groups of forty mice each were placed on the stock diet to which was added powdered whole liver, the latter forming 10 per cent of the total diet. All of them were given injections of 5 per cent suspension of sodium caseinate according to the routine procedure.

Two mice were killed at successive intervals after 32, 42, 56, 62, 66, 78 and 105 injections. After 66 injections early evidences of amyloidosis were noted.

In this group no histologic alterations were apparent in the spleen until after the forty-second injection. At this time the spleen showed the appearances noted in group 1 before the thirty-second injection. After 56 injections the appearance was that of the spleen of group 1 at the thirty-second injection stage, while not until 66 injections had been administered did definite amyloid, in amounts approximately equal to that seen in group 1 after the forty-second injection, appear in the spleen.

Likewise, no hepatic changes were apparent until after the fifty-sixth injection, when the appearance was that observed in animals of group 1 after the thirty-second injection. After 66 injections had been given, the sections of the liver presented the picture of animals of group 1 after 42 injections. No renal changes, except for a moderate glomerular endothelial cell hyperplasia, were apparent even after the sixty-sixth injection.

4. *Stock Diet with the Addition of Ground Beef Meat*.—To the stock diet ground beef meat, forming 10 per cent of the total diet, was added and fed to a

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27. Strasser: Ztschr. f. d. ges. exper. Med. **36**:381, 1923. Morgenstern.<sup>25</sup>

28. The diet is based on the recommendation of H. Steenbock (Science **58**:449, 1923) for a well balanced diet.

29. The preparation of desiccated whole liver is so made that 1 Gm. is obtained from 8 Gm. of raw fresh liver.

group of forty albino mice. The inoculations with 5 per cent nutrose suspension were performed in the usual routine manner. After 32, 36, 42, 56, 62, 66, 78 and 105 injections, respectively, two mice were killed and studied.

Amyloidosis was noted after 32 injections, and with successive injections increasing amounts of amyloid was observed.

The findings here corresponded to those obtained in group 1 (stock diet).

5. *Stock Diet with the Addition of Liver Extract No. 343.*<sup>30</sup>—Fifty albino mice were placed in this group. The diet consisted of 1 part of powdered liver extract

TABLE 3.—*Influence of Diet and Dietary Factors and Ingested Chemical Substances on the Production of Amyloidosis\**

Number of Injections	Stock Diet	Adequate Diet	Stock Diet and Liver Meal	Stock Diet and Ground Beef	Stock Diet and Liver Extract No. 343	Stock Diet and Liver Extract with Ferrous Ammonium Citrate	Stock Diet and Cholesterol	Stock Diet and Sodium Acid Phosphate	Stock Diet and Sodium Bicarbonate
11	..	..	..	..	..	0	..	..	..
15	0	..	..	..	..	..	..	0	..
18	..	..	..	..	..	..	..	..	..
20	0	..	..	..	..	..	..	0	..
21	..	..	..	..	..	..	..	0	..
23	..	..	..	..	..	0	..	..	..
24	..	..	..	..	0	0	0	..	..
27	..	..	..	..	..	..	..	..	0
30	..	..	..	..	..	..	..	..	..
31	..	..	..	..	0	..	0	..	..
32	+	..	0	+	..	..	..	..	..
33	..	0	..	..	..	..	..	..	..
35	..	..	..	..	..	..	..	..	..
36	++	..	..	+	..	..	++	..	..
39	..	..	..	..	0	..	..	..	..
42	++	0	0	++	..	..	..	..	..
43	..	..	..	..	..	..	++	..	..
46	..	..	..	..	+	..	..	..	..
47	..	0	..	..	..	..	..	..	..
50	..	..	..	..	..	..	..	..	..
56	+++	0	±	++	++	..	..	..	..
57	..	..	..	..	..	..	..	..	..
62	..	±	±	+++	..	..	..	..	..
66	..	..	+	++++	..	..	..	..	..
78	..	..	+	++++	..	..	..	..	..
80	..	+	..	..	..	..	..	..	..
89	..	++	..	..	..	..	..	..	..
105	..	..	++	++++	..	..	..	..	..

\* 0 indicates no amyloidosis; ±, precursory changes of amyloidosis; +, early amyloidosis; ++, moderate amyloidosis; +++, moderately advanced amyloidosis; +++, advanced amyloidosis.

no. 343 and 24 parts of the stock diet. Two or three mice were killed after 24, 31, 39, 46 and 56 injections.

No amyloidosis was noted after 24, 31 and 39 injections. Following the forty-sixth injection, there were moderate amyloid changes in the spleen and early amyloid changes in the kidneys.

6. *Stock Diet with the Addition of Liver Extract with Ferrous Ammonium Citrate.*<sup>31</sup>—Fifty albino mice were placed in this group. One part of liver extract with ferrous ammonium citrate to 29 parts of the stock diet was fed to the mice. Injections of a 5 per cent solution of sodium caseinate were given in the usual manner.

30. One gram of this preparation is prepared from 25 Gm. of raw fresh liver.

31. One gram of this preparation is prepared from 30 Gm. of raw fresh liver and 0.17 Gm. of ferrous ammonium citrate.



In these two studies, approximately equivalent amounts of the products obtained from raw fresh liver were added to the stock diet.

The mice in this group fared badly. A rapid decline in their health occurred, as evidenced by their appearance, behavior and appetite, and by the high mortality. Three mice were killed after 11 injections, and two mice after 24 injections. After the latter period, none survived. *The killed mice showed no evidence of amyloidosis.*

It was decided to repeat this experiment with another group of forty mice, in order to study the possibility of infection or some other unknown factor as a cause of the early death of the mice. However, the results were the same. After 23 injections, only two mice were alive, and these showed no amyloidosis when killed.

7. *Stock Diet with the Addition of Cholesterol.*—A diet consisting of 0.8 Gm. of powdered cholesterol (Eimer and Amend) to 100 Gm. of the stock diet<sup>32</sup> was fed to a group of fifty mice. The mice were killed after 24, 31, 39 and 46 injections.

There was no evidence of amyloidosis after 24 and 31 injections. Following the thirty-ninth injection moderate splenic amyloid and slight perivascular intracellular hepatic amyloid were observed. After the forty-sixth inoculation moderate perifollicular and intrapulp splenic amyloid and slight perivascular intracellular hepatic amyloid could be demonstrated.

8. *Stock Diet with the Addition of 1 or 2 Per Cent of Sodium Acid Phosphate.*—Two groups of forty mice each were given the stock diet, with the addition of sodium acid phosphate, one set receiving 1 per cent of the total food in the form of the acid salt, the other 2 per cent. In the former group, none of the mice survived longer than twenty-six days on this diet, during which time 20 injections were given; in the latter group all were dead after 15 injections and a period of twenty-one days on the diet. None of the mice killed after 15 and 20 injections showed evidences of amyloidosis.

A control group of twenty uninoculated mice were placed on the diet containing 2 per cent of the acid salt. These mice apparently thrived on this diet. At the end of a period of two hundred days, they were still alive.

The urine was repeatedly tested with litmus and was found acid.

9. *Stock Diet with the Addition of 1 or 2 Per Cent Sodium Bicarbonate.*—Two groups of forty mice received the stock diet, which contained sodium bicarbonate. In the case of one group, 1 per cent of the alkali salt was added to the diet; in the second group, 2 per cent of the salt. None of the mice survived more than 27 injections or after receiving the diet for thirty-six days. The urine became alkaline to litmus within seven days. The control group of twenty mice, which did not receive the inoculations, still lived at the end of two hundred days.

No evidence of amyloidosis was noted in the mice that were killed.

#### COMMENT

On the ordinary stock diet, definite amyloidosis was produced in all albino mice after from 30 to 35 injections. The condition became progressively more marked with the increasing number of injections.

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32. Cholesterol was added in amounts approximating those used by Jaffé<sup>3</sup> in order to repeat his work and to compare its influence with the substances utilized in our investigation.

On a highly adequate diet, the mice failed to show any evidences of amyloidosis until they had received 62 injections. Furthermore, it was only after 81 injections that they showed definite signs.

This demonstrates the highly preventive character of such a diet, and the important rôle that diet may play in this disease. It is possible that the failures of some of the investigators in the production of amyloidosis in albino mice and in other animals may have been due to their lack of consideration of the rôle of dietary factors.

The addition of powdered whole liver to the stock diet also caused significant retardation. The first suggestive signs of amyloid formation appeared after the fifty-eighth injection. Such an effect was not obtained when ground beef meat, liver extract no. 343, liver extract with ferrous ammonium citrate or cholesterol was included in the stock diet in place of the desiccated whole liver.

That the marked delay in the formation of amyloid in mice receiving the powdered whole liver is not due to the inclusion of animal meat or protein in the diet is evidenced by the absence of such protection in the mice receiving the addition of ground beef meat. Consequently, it is due to some other factor or factors in the whole liver, which, furthermore, are not present in liver extract no. 343 and liver extract with ferrous ammonium citrate. The rich vitamin content of liver may be the determining factor, in view of the retarding influence of vitamins A and B (part IV). Another possibility is the presence of an active principle in the liver. The possible manner in which the liver exerts its beneficial effect is discussed in part II.

The effect of the addition of a weak acid or base to the stock diet cannot be studied satisfactorily in view of the early death of the mice. However, such additions do not accelerate the formation of amyloid, but in conjunction with the inoculations seem to impair the health of the animals.

#### IV. INFLUENCE OF VITAMINS ON THE PRODUCTION OF AMYLOIDOSIS

The possibility that the qualitative nature of the diet might influence this process was considered worthy of study. In the preceding section evident retardation of the development of amyloidosis was noted with certain diets. Furthermore, delay in the production of amyloidosis occurred in the group whose stock diet included desiccated powdered whole liver. Ordinarily, on the so-called stock diet, which consisted of powdered whole milk and finely ground white bread, definite amyloidosis set in after from 30 to 35 injections of a 5 per cent suspension of sodium caseinate. With the diets contributing toward the retardation of the condition, amyloidosis did not appear until much later, sometimes not until after 66 injections. Each of the diets mentioned had one thing in common. They all contained an abundance of vitamins A, B

complex and D. The present investigation was undertaken with the object of determining if a single vitamin or a specific combination of them was responsible for the effect.

#### METHODS AND RESULTS

The experiment was carried out in two parts. The first consisted of the use of a synthetic diet with the addition of single vitamins and various combinations of them. In the second study, the vitamins were added to the stock diet consisting of powdered whole milk and ground white bread.

*Part A: Synthetic Diet and Vitamins.*—Sixteen groups of albino mice were formed, each containing thirty animals. The synthetic diet was composed of 31 per cent commercial casein, 40 per cent corn-starch, 22 per cent lard and 7 per cent salt mixture.<sup>33</sup> The diets fed the group were as follows:

- (A) Synthetic diet<sup>34</sup>
- (B) Synthetic diet plus 10 per cent butter fat (vitamin A)
- (C) Synthetic diet plus 5 per cent powdered yeast (vitamin B)
- (D) Synthetic diet plus orange juice (60 cc. per 100 cc. of tap water) (vitamin C)
- (E) Synthetic diet plus 1 per cent viosterol (vitamin D)
- (F) Synthetic diet plus butter fat plus yeast (vitamins A and B)
- (G) Synthetic diet plus butter fat plus orange juice (vitamins A and C)
- (H) Synthetic diet plus 5 per cent cod liver oil (vitamins A and D)
- (I) Synthetic diet plus butter fat plus yeast plus orange juice (vitamins A, B and C)
- (J) Synthetic diet plus cod liver oil plus yeast (vitamins A, D and B)
- (K) Synthetic diet plus cod liver oil plus orange juice (vitamins A, D and C)
- (L) Synthetic diet plus yeast plus orange juice (vitamins B and C)
- (M) Synthetic diet plus yeast plus viosterol (vitamins B and D)
- (N) Synthetic diet plus yeast plus viosterol plus orange juice (vitamins B, C and D)
- (O) Synthetic diet plus viosterol plus orange juice (vitamins C and D)
- (P) Synthetic diet plus cod liver oil plus yeast plus orange juice (vitamins A, B, C and D)

The routine procedure for the production and study of amyloidosis which was described in part I was carried out. The animals were killed and studied at intervals as shown in table 4. The duration of the study was limited by the failure of the animals to survive when subjected to injections of sodium caseinate suspension while on the synthetic diet. Some of the groups succumbed so rapidly that these phases of the experiment were repeated.

*Part B: Stock Diet and Vitamins.*—The failure of maintenance of mice on the synthetic diet for a sufficient period of time for satisfactory study of the influence of vitamins on amyloidosis led to a similar study on mice given what is apparently

33. Mitchell, H. S., and Mendel, L. B.: *Am. J. Physiol.* **58**:211, 1921.

34. The diet is based on the standard diets for mice described by Mitchell and Mendell<sup>33</sup> and by Beard (*Am. J. Physiol.* **75**:645, 1926).

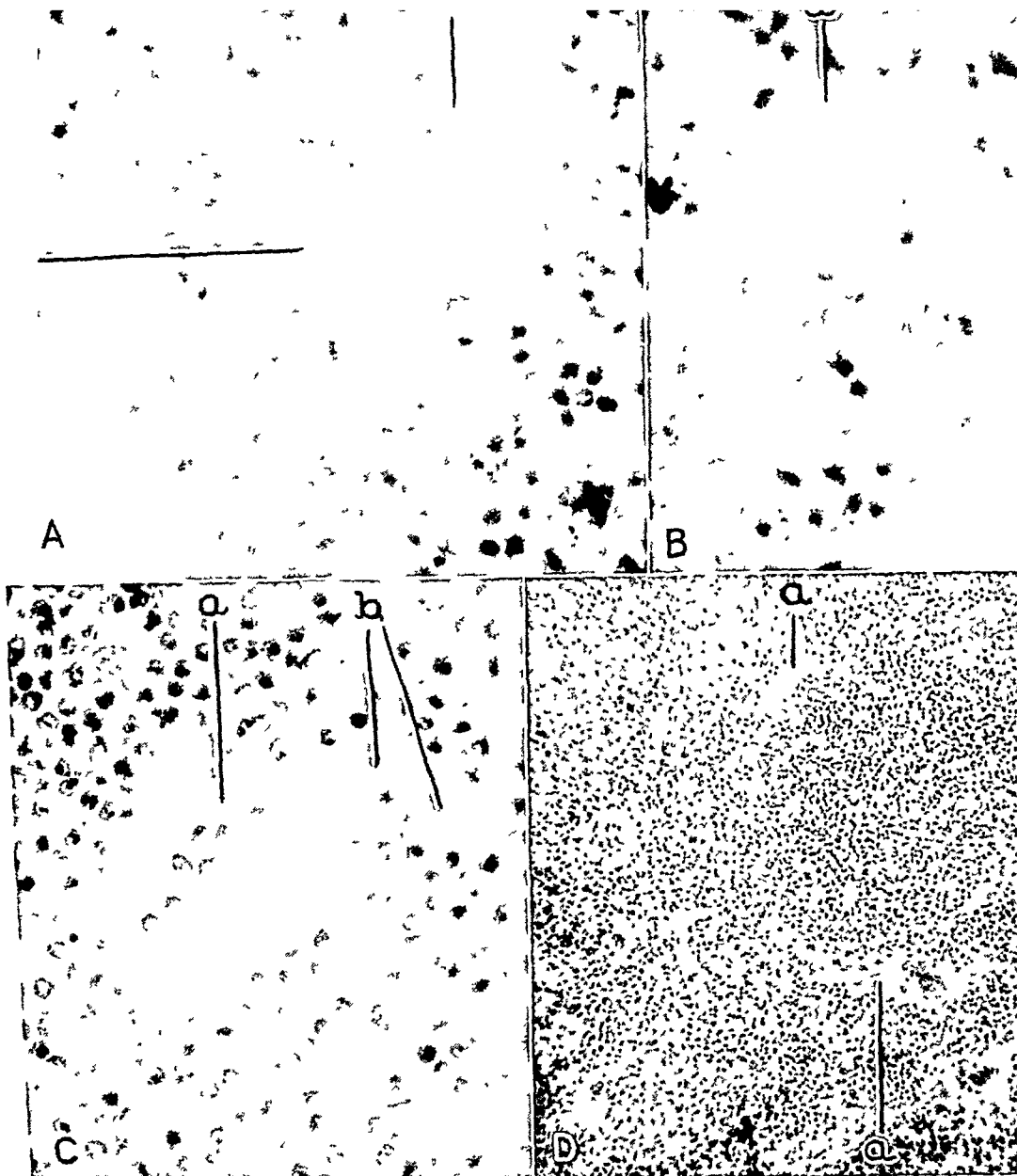


Fig. 1.—Microscopic appearance of the spleen after injections of sodium caseinate. *A*, the spleen after 36 injections of nutrose, showing 4 uninucleated (*a*) and 1 multinucleated macrophage (*b*) in the pulp sinus. The cytoplasm of the former was tinted deep pink-orange, that of the latter deep red. The latter cell shows poorly defined cellular and nuclear outline and some nuclear fragmentation. This stage just precedes the first appearance of extracellular amyloid;  $\times 400$ . *B*, the spleen after 46 injections of sodium caseinate, showing a much enlarged, poorly outlined, multinucleated macrophage (*a*) in the splenic pulp. The cell outline can clearly be seen only in a few places; elsewhere along the periphery, it merges with adjacent thickened reticular groundwork amyloid fibers;  $\times 400$ . *C*, the spleen after 54 injections of sodium caseinate, showing a mass of homogeneous amyloid within a pulp sinus, distending it and fusing at its upper end with an adjacent thickened sinus wall (*a*). Two very faintly outlined macrophages (*b*), their nuclear outlines only visible, can be seen in this zone;  $\times 400$ . *D*, the spleen after 42 injections of sodium caseinate, showing thickened strands (*a*) of reticular groundwork fibers in the perifollicular zone (fig. 2*A*). These strands, as well as those in the following photomicrographs, assumed the specific pink-red to red tint with congo red;  $\times 80$ .

TABLE 4.—*Summary of Pathologic Findings in Albino Mice on Synthetic Diet and Combinations of Vitamins\**

Number of Injections	Synthetic Diet Plus												
	Synthetic Diet (Group A)	Vitamin A (Group B)	Vitamin B (Group C)	Vitamin C (Group D)	Vitamin D (Group E)	Vitamins A and B (Group F)	Vitamins A and C (Group G)	Vitamins A and D (Group H)	Vitamins A, B and C (Group I)	Vitamins A, B and D (Group J)	Vitamins A, C and D (Group K)	Vitamins B and C (Group L)	Vitamins B and D (Group M)
18	0	+	0	0	0	0	0	0	0	0	0	0	0
20	0	+	0	0	0	0	0	0	0	0	0	0	0
21	0	+	0	0	0	0	0	0	0	0	0	0	0
23	0	+	0	0	0	0	0	0	0	0	0	0	0
24	0	+	0	0	0	0	0	0	0	0	0	0	0
25	0	+	0	0	0	0	0	0	0	0	0	0	0
26	0	+	0	0	0	0	0	0	0	0	0	0	0
27	0	+	0	0	0	0	0	0	0	0	0	0	0
31	0	+	0	0	0	0	0	0	0	0	0	0	0
33	0	+	0	0	0	0	0	0	0	0	0	0	0
34	0	+	0	0	0	0	0	0	0	0	0	0	0
36	0	+	0	0	0	0	0	0	0	0	0	0	0
42	0	+	0	0	0	0	0	0	0	0	0	0	0
45	0	+	0	0	0	0	0	0	0	0	0	0	0
53	0	+	0	0	0	0	0	0	0	0	0	0	0
67	0	+	0	0	0	0	0	0	0	0	0	0	0

\* 0 indicates no amyloidosis; ±, doubtful amyloidosis; +, early amyloidosis; ++, moderate amyloidosis.

TABLE 5.—*Summary of Pathologic Changes in Albino Mice on Stock Diet and Combinations of Vitamins\**

Number of Injections	Stock Diet Plus							
	Stock Diet (Group A)	Vitamin A (Group B)	Vitamins A and D (Group C)	Vitamin D (Group D)	Vitamin B (Group E)	Vitamins A and B (Group F)	Vitamins B and D (Group G)	Vitamins A, B and D (Group H)
24	0	0	0	+	0	0	+	0
30	0	0	+	0	++	0	+	0
32	+	0	0	0	0	0	0	0
35	+	+	0	0	0	0	+++	0
36	++	0	0	0	0	0	0	0
42	+++	0	0	0	0	0	0	0
43	0	++	+++	+	+++	0	+++	+
50	0	0	++	+++	+++	0	+++	0
56	++++	0	0	0	0	0	+++	0
57	0	+++	0	0	0	0	0	0
65	0	++++	+++	0	0	0	0	0
75	0	0	+++	0	++++	+++	+++	0

\* 0 indicates no amyloidosis; +, early amyloidosis; ++, moderate amyloidosis; +++, moderately advanced amyloidosis; +++++, advanced amyloidosis.

a more satisfactory diet, namely, our so-called stock diet. This diet consisted of 40 per cent ground white bread and 60 per cent powdered whole milk.

Eight groups of twenty-eight albino mice each were formed. The diets fed were as follows:

(A) Stock diet of powdered whole milk and ground bread

(B) Stock diet plus 0.5 per cent carotene solution<sup>35</sup> (vitamin A)

35. von Euler, B.; von Euler, H., and Hellström, H.: *Biochem. Ztschr.* **203**: 370, 1928. von Euler, B.; von Euler, H., and Karrer, P.: *Helv. Chim. Acta* **12**: 278, 1929. Moore, T.: *Biochem. J.* **23**:807, 1929; **24**:692, 1930; **25**:275, 1931. Collison, D. L.; Hume, E. M.; Smedley-Maclean, I., and Smith, H. H.: *ibid.* **23**: 634, 1929. Hume, E. M., and Smith, H. H. *ibid.* **22**:504, 1928. Capper, N. S.: *ibid.* **24**:980, 1930.

- (C) Stock diet plus 5 per cent cod liver oil (vitamins A and D)
- (D) Stock diet plus 1 per cent viosterol (vitamin D)
- (E) Stock diet plus 5 per cent brewer's powdered yeast (vitamin B complex)
- (F) Stock diet plus carotene solution plus powdered yeast (vitamins A and B)
- (G) Stock diet plus powdered yeast plus viosterol (vitamins B and D)
- (H) Stock diet plus cod liver oil plus powdered yeast (vitamins A, D and B)

The results are given in table 5.

#### COMMENT

The albino mice on the synthetic diet, without or with one or more vitamins, did not survive a sufficient period to determine the influence of the accessory food substances on the production of amyloidosis. Practically all of them died before 30 to 35 injections were given. The combination of the injections of sodium caseinate suspension and the unsuitable diet made maintenance of life impossible. The control groups (uninoculated) lived from sixty-two to one hundred and eleven days, whereas the groups given injections died within from thirty-one to fifty-four days. Only two groups (one receiving vitamins A and B and one vitamins A, B and C) lived beyond this period, but did not show amyloidosis after 42 and 67 injections, respectively. Furthermore, although the synthetic diets were inadequate under the established conditions, there was no evidence of early amyloidosis and therefore of an acceleration of the onset of this disease.

Of the mice fed the stock diet, most groups showed amyloidosis after from 30 to 35 injections. However, animals the diet of which contained vitamins A and B showed definite retardation of the production of amyloidosis. The group receiving vitamins A and B showed no amyloid after 75 injections; the group fed vitamins A, B and D revealed early amyloidosis after 43 injections, none after 50 injections and again slight evidence after 57 injections. The early changes after 43 injections are explained by the fact that the mice selected for examination appeared ill and in poor physical condition. This illustrates the individual variability in susceptibility to amyloidosis.

Various studies have been made in animals deprived of vitamins A and B, and from our incomplete knowledge of the possible rôle of these vitamins and their effect on tissues we may hypothesize as to the relationship of amyloidosis.

McCarrison<sup>36</sup> noted that lack of vitamin A produced functional and degenerative changes in every tissue of the body, including the spleen, intestines, liver and kidneys.

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36. McCarrison, R.: *Brit. M. J.* **1**:177, 1919; *Indian J. M. Research* **7**:167 and 188, 1919.

Wolbach and Howe <sup>37</sup> observed that in man and in the rat, deprivation of vitamin A results in striking epithelial changes. Normal epithelium of the respiratory tract, gastro-intestinal tract, eyes, para-ocular glands and genito-urinary tract are replaced by stratified keratinizing epithelium. Wason <sup>38</sup> and Yudkin and Lambert <sup>39</sup> described such changes in the eyes. Mori <sup>40</sup> noted this change in the mouth, larynx and trachea and the glands.

Findlay and MacKenzie <sup>41</sup> showed that in rats deprived of vitamin A there occur almost complete replacement of the hematopoietic tissue by fibrous tissue and a marked decrease in leukocytic cells.

The importance of vitamin A in the diet for regeneration of blood was reported by Koessler and his associates.<sup>42</sup> Damianovich and his co-workers <sup>43</sup> showed that absence of vitamin A or B in the diet produces a progressive anemia. Abderhalden <sup>44</sup> reported a marked decrease in red blood cells in pigeons deprived of vitamin B. Cramer and his associates <sup>45</sup> stated that lack of vitamin B causes atrophy of the lymphoid tissue throughout the body and lymphopenia in the blood.

Dutcher <sup>46</sup> asserted that vitamin B is a metabolic stimulant. Its absence caused a decrease of the catalase content of the tissues, a depression of tissue oxidation and an accumulation of toxic metabolic products. Bickel <sup>47</sup> contended that absence of vitamin B causes a progressive loss of the power of synthesis and storage of digestive products. Findlay <sup>48</sup> expressed the belief that vitamin B is necessary in the synthesis of nucleic acids. Rohr <sup>49</sup> found that in the absence of vitamin B there occurs a decrease of respiratory activity in the kidney, liver, brain and muscle. Jorstad <sup>50</sup> showed that large additions of vitamin A stimulate the formation and growth and prolong the life of fibroblasts, endothelial cells and other cells that are attracted to the local area

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37. Wolbach, S. B., and Howe, P. R.: *Arch. Path.* **5**:239, 1928.

38. Wason, I. M.: *J. A. M. A.* **76**:908, 1921.

39. Yudkin, A. M., and Lambert, R. A.: *Proc. Soc. Exper. Biol. & Med.* **19**:375, 1922.

40. Mori, S.: *Bull. Johns Hopkins Hosp.* **33**:357, 1922; *Am. J. Hyg.* **3**:99, 1923.

41. Findlay, G. M., and MacKenzie, R. D.: *J. Path. & Bact.* **25**:402, 1922.

42. Koessler, K. K.; Maurer, S., and Loughlin, R.: *J. A. M. A.* **87**:476, 1926.

43. Damianovich, H.; Branchi, A., and Savazzina, L. A.: *Compt. rend. Soc. de Biol.* **88**:377, 1923.

44. Abderhalden, E.: *Klin. Wchnschr.* **1**:160, 1922.

45. Cramer, W.; Drew, A. H., and Mottram, J. C.: *Lancet* **1**:963, 1921; **2**:202, 1921.

46. Dutcher, R. A.: *J. Biol. Chem.* **36**:63 and 551, 1918; *Proc. Nat. Acad. Sc.* **6**:10, 1920.

47. Bickel, A.: *Klin. Wchnschr.* **1**:110, 1922.

48. Findlay, G. M.: *J. Path. & Bact.* **24**:175, 446 and 454, 1921.

49. Rohr, F.: *Ztschr. f. Physiol. Chem.* **129**:248, 1923.

50. Jorstad, L. H.: *J. Exper. Med.* **42**:221, 1925.

where coal tar has been injected. According to him, vitamin A protects the cells against the toxic action of the tar and retards their disintegration. Burrows and Jorstad<sup>51</sup> asserted that for growth of body cells a certain substance or substances, believed by them to be identical with vitamin B, were necessary. Furthermore, for extracellular formation in general and for growth and proper structure and function of life, a certain soaplike substance is necessary. This substance is identical with vitamin A. To prevent depletion of this valuable material vitamin A must be supplied.

Summarizing, we may say that deprivation of vitamins A and B leads to functional and structural changes in various organs, including the spleen, liver and kidney, the hematopoietic tissue and the lymphoid tissue. In addition, various metabolic disturbances are produced, such as decreased oxidation, loss of the power of synthesis and storage of digestive products and nucleic acids. Furthermore, there occurs a decreased response to the introduction and elimination of toxic material with early degeneration of the fibroblasts, endothelial cells and other cells. Lastly, inadequate growth and improper general bodily functions result.

These investigations deal with disturbances produced by absence of the vitamins from the diet. It is possible that the supply of these two vitamins in large amounts may cause not only a restoration to optimal normal conditions, but also an enhancement of all of these normal physiologic processes.

In part I a conception of the possible mechanism for the production of amyloidosis was stated. The nature of this condition was considered to be one of a severe and prolonged disorder of endogenous protein metabolism. The abnormal destruction of tissue protein resulted in the production of chemical products which were not readily metabolized to a form which would be utilized or eliminated easily. Failure of disposal of these at a rate commensurate with their formation resulted in their deposition in the tissues.

Carrying this idea further, it may be said that an animal under such conditions is under a strain, and must mobilize all its resources for coping with the amyloid precursor. It is successful in meeting the disturbance and in preventing or retarding amyloidosis to the extent to which it can obtain maximal and optimal function of all the available agents.

An explanation which may account for the retarding influence of vitamins A and B on amyloidosis may be suggested. Vitamins A and B play an important rôle in certain general metabolic processes, and in the satisfactory function and growth of organs (the spleen and liver)

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51. Burrows, M. T., and Jorstad, L. H.: *Am. J. Physiol.* **77**:24, 1926.



and tissues (the hematopoietic and mesenchymal tissue). Amyloidosis is a metabolic disease in which the reticulo-endothelial cells participate. Consequently, an animal will deal more successfully with a disorder when it receives adequate amounts of food factors which are necessary

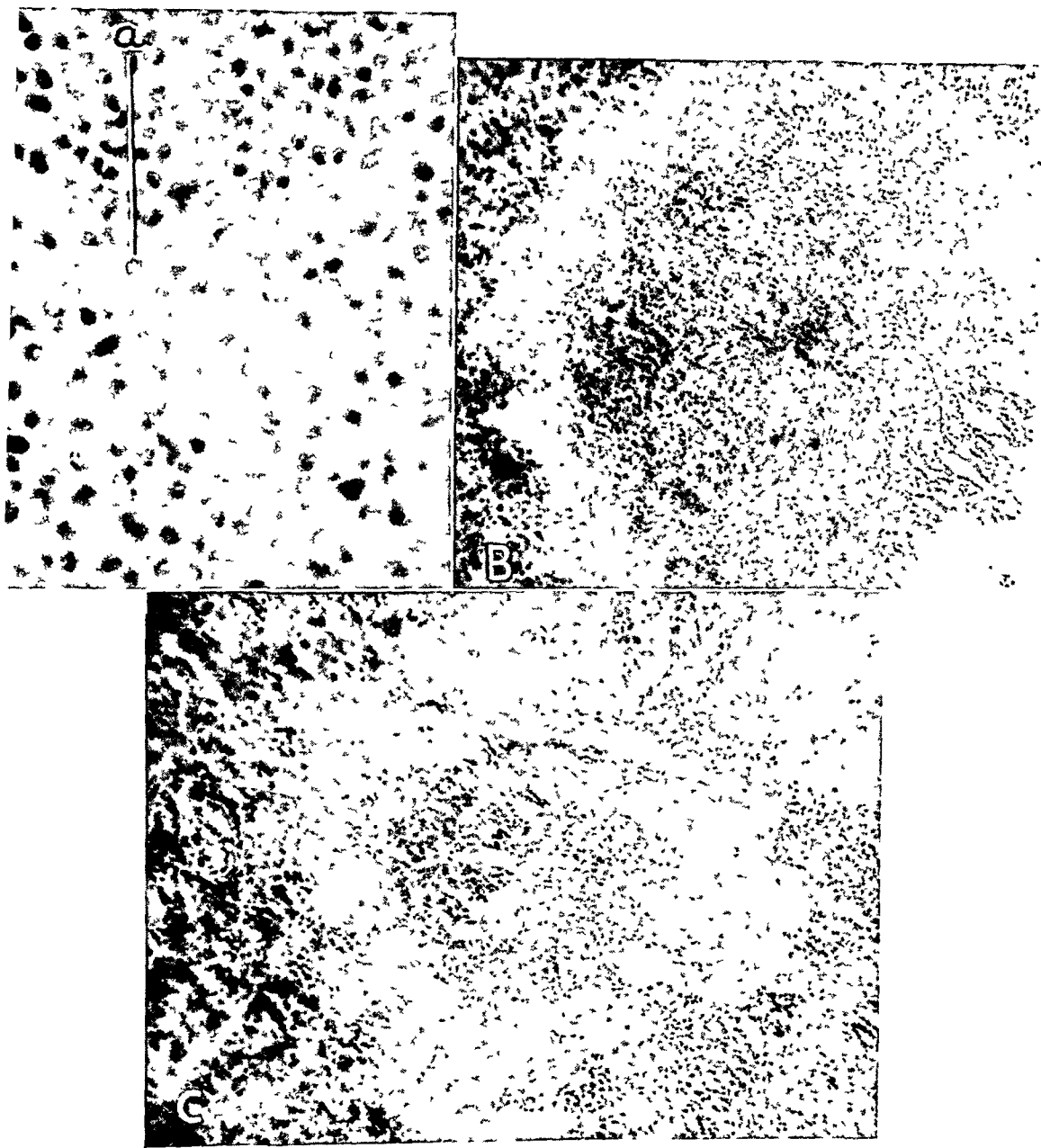


Fig. 2—Microscopic appearance of the spleen after injections of sodium caseinate. *A*, a higher power photomicrograph of figure 1 *D*, showing the relation of the thickened amyloid fibers (*a*) to the pulp lymphocytic cells;  $\times 400$ . *B*, the spleen after 48 injections of sodium caseinate, showing a perifollicular ring of moderate amount of homogeneous amyloid. There is no follicular compression or atrophy;  $\times 80$ . *C*, the spleen after 56 injections of sodium caseinate, showing a vastly increased amount of amyloid, still arranged perifollicularly, with marked compression atrophy of the follicle;  $\times 80$ .

for satisfactory metabolism and function of tissues concerned in this disturbance.

A minimal number of injections are required, and a certain period of time must elapse before amyloidosis sets in even with inadequate diets or with diets lacking one or all vitamins. It seems, further, that amyloid-like or precursory amyloid changes may be taking place without more than suggestive fixed and wandering cellular morphologic changes in organs being apparent. When, however, an apparent threshold, possibly the resultant of the defense-enhancing factors for the reticular system and the metabolic disturbance, is exceeded, the amyloid suddenly becomes visible in large amounts without going through the progressive phases of its usual morphogenesis. This view seems indicated from the results of this experiment (table 2, group 6). Such a view may further explain the well known individual variability shown by animals.

#### SUMMARY

Amyloidosis can be produced in all albino mice by subcutaneous or intramuscular injections of a 5 per cent aqueous suspension of sodium caseinate.

The earliest amyloid appears within the fixed and wandering cells of the reticular system. As these cells disintegrate, extracellular amyloid appears, grows in amount and finally replaces the parenchyma of the organ involved.

Amyloidosis is probably the result of an endogenous protein metabolic disturbance. When the rate of formation of these catabolic products exceeds the ability of the tissues to dispose of them, amyloid appears.

With the present technic, amyloidosis cannot be produced in albino rats.

Except in albino mice showing precursory or very early evidences of amyloidosis, no spontaneous resorption of amyloid in definite cases of amyloidosis was observed. Albino mice given a preparation of powdered whole liver in their diet showed resorption only when the degree of amyloidosis was no more than moderate. No retrogression of the disease was noted in advanced cases.

Comparative studies indicate that a well balanced, thoroughly adequate diet exercises a retarding influence on the production of amyloidosis.

The addition of a preparation of desiccated powdered whole liver to the stock diet results in delay of the formation of amyloidosis.

Inadequate or deficient diets do not accelerate the development of amyloidosis.

Mice fed a synthetic and the so-called stock diet to which vitamins A and B were added showed definite evidence of retardation of the production and formation of amyloidosis.

# General Review

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## THE LUNGS AND THE MACROPHAGE SYSTEM

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NEW YORK

Reactions to infection depend on the nature of the infectious material (the antigen), the species of the animal infected, the route of infection and also on whether the infection occurred for the first time (normergic or allergic reaction).

The remarkable phenomenon in the process of local reaction to infection is the rapid accumulation in the affected area of cells which previously were present in scant numbers. The nature and origin of this cellular "deluge" has been and remains the bone of contention among investigators.

With the inauguration of the conception of "cellular pathology" the theory of the specificity of cells according to the germinal layer has not been appreciated. For example, in order to explain the occurrence of different varieties of tumors, Virchow devised the theory of metaplasia whereby "one well characterized tissue transforms itself into another equally well characterized but morphologically and functionally different." He disputed that carcinoma originates from epithelial tissues, and up to the end of the century he supported the erroneous observation that Gaucher's disease is a primary epithelioma of the spleen whereby the large "epithelial" cells of which the splenic tumor is made up originate through metaplasia of the connective tissue of the spleen. Pathologists were formerly opposed to Cohnheim's conception of the diapedesis of cells in inflammation, believing that the white blood corpuscles always originate in situ. They likewise disputed the teaching of the "proclivities" of the macrophage, which appeared to them to be rather grotesque.

Cells of which an inflammatory exudate is made up consist of red and white corpuscles, namely, polymorphonuclear leukocytes (granulocytes) and mononuclear cells consisting of lymphocytes, monocytes and macrophages. Whereas the immediate source of the erythrocytes and the granulocytes is commonly accepted as being the circulating blood, the genesis of the large mononuclear phagocytes is debatable.

Metchnikoff, who was the first to stress the rôle of the mesenchyma and to identify the mesodermal cells as the essential elements in the

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field of inflammation, maintained that they originate from the local connective tissue, from the endothelium lining the capillaries and from the circulating blood. Mallory was among the earlier observers who adhered to the conception that the large mononuclear phagocytes always originate from the endothelial cells lining the blood vessels, and accordingly he designated them as endothelial leukocytes. This theory was advocated particularly by Haythorn, Permar, and by Foot, and it dominated the teaching of pathology in this country for more than a quarter of a century. Sabin and her associates, basing their studies on supravital technic, devised the theory of two genetically and functionally different strains of phagocytic cells: the monocyte, which evolves from the reticular cell, and the clasmatocyte, which is of endothelial origin. Aschoff traced the origin of the macrophages to the local connective tissue (histiocytes, or tissue cells), while Maximow produced evidence to show that the polyblast or macrophage in inflammation has a double origin: hematogenous and histogenous. In the common loose connective tissue and in the serous membranes the resting and wandering cells are: adventitial cells, the pericytes and the clasmatocytes. Maximow included in the group of hematogenous cells the monocytes of the blood and also the lymphocytes, which according to his experiments, transform themselves into macrophages. This concept was originally expressed by Metchnikoff, who wrote: "*Si les lymphocytes mêmes ne sont encore point des phagocytes, ils le deviennent bientôt après s'être transformé en cellules épithélioïdes.*" ("If the lymphocytes are not yet phagocytes, they soon become so after having been transformed into epithelioid cells." Lewis and Lewis concluded from their studies on tissue cultures that the mononuclear monocytes, the macrophages and the epithelioid cells are related to temporary functional variations. These authorities could find no definite evidence of the transformation of the lymphocyte into a monocyte. They assumed that the epithelioid cells of tuberculous lesions are derived either directly or indirectly from the mononuclears of the blood. Von Möllendorff, in recent publications, claimed that the fibroblast, too, is capable under proper circumstances of transforming itself into a macrophage, but this was energetically contradicted by Maximow in articles which were published shortly before his death.

Before presenting the discussion on the mesenchymal reactions of the lungs, it may be well to review briefly the opinions regarding the normal histology of the air sacs.

#### STRUCTURE OF THE PULMONARY PARENCHYMA

The structure of the respiratory portion of the lungs was investigated by numerous authorities, particularly by Miller, who summarized his views in the Harvey Society Lectures of 1924 and 1925. Whether the

air sacs are directly connected with the terminal bronchioles or whether there exists between them an interposed cavity, an atrium, as was constructed by Miller, is irrelevant to the present report.

The alveolar wall was described by von K  lliker and Schaffer as being a homogeneous membrane containing no collagenous fibers but endowed with elastic fibers and harboring capillaries. Von M  llendorff and Russakoff described the septums as being composed of a fine network of reticulum fibers which rarely insinuate themselves between the capillaries and the lining epithelium. The reticulum fibers were looked on by Ors  s as being collagenic. The same observer discriminated between two varieties of elastic fibers: respiratory, which are made up of coarse fibers not related to the capillaries, and intercapillary, the fine fibers of which are interwoven with the capillaries. A few histologists found a basal membrane covering the septums, the supporting substance of which contained a sparse number of mesenchymal cells.

Of particular interest is the question relating to the cells lining the air sacs, the so-called alveolar epithelium. Von K  lliker, whose opinion was shared by most histologists, found that the air sacs are lined by large non-nucleated plates interspersed with groups of small nucleated epithelial cells. "With the general acceptance of K  lliker's description of the alveolar epithelium," wrote Miller, "all investigation of the alveolar epithelium practically ends, and his illustrations are frequently reproduced." In a recent review Miller stated that "in the normal lung, the epithelium lining the alveolar wall is made up of thin, flattened, nucleated squam  e which are closely applied to the alveolar wall, and it is a continuous epithelium."

Bloom, in Maximow's textbook of histology, stated that "the details of the structure of the respiratory portion of the lung have been only partially elucidated." "There can be little doubt," he stated further, "that the so-called 'nucleated alveolar epithelial cells' of the older authors are composed of certain pericapillary cells—probably also the endothelial cells of the capillaries and even the red blood corpuscles within these vessels."

The problem, which superficially appears to be that of cytology, is in reality one of the physiology and pathology of the lungs. This will be elaborated in the following discussion.<sup>1</sup>

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1. "The problem of general physiology," wrote Claude Bernard, "is centered around the histologic elements. The physiologist will understand the mechanism of life and will be in a position to influence it scientifically when the circumstances which influence the cells in their intimate organic medium have been established." (*Rapport sur le progr  s de la physiologie g  n  rale*, Paris, J. B. Bailli  re et fils, 1863.)

## THE DEFENSE RÔLE OF THE LUNGS

Observers in the past expressed the hypothesis that the function of the lungs is probably not limited to gaseous exchange. A study made by Fort in 1867 was to the effect that these organs display characteristics of secretory organs and accordingly he used the term pulmonary gland, which was later accepted by Roger and by Aschoff. The function of the lungs is probably complex. From the point of view of circulation they are the point of convergence of the body fluids, and material which happens to invade the circulation invariably reaches the lungs. Then, they are the single visceral organ which is in direct contact with the outside world. They are, therefore, liable to receive dust, bacteria and other matters directly from the air. It is remarkable that in spite of the exposed position the pulmonary parenchyma usually contains no bacteria. Moreover, investigations have shown that when pathogenic micro-organisms are experimentally introduced into the respiratory portion of the lungs by way of the trachea, they rapidly disappear, in most instances without causing disease.

What is the mechanism whereby the lungs maintain their sterility in normal conditions? How do they react in instances of inflammation?

The defensive rôle of the organism in general is attributed to the connective (mesenchymal) tissues and particularly to a variety of cells which are derived from the embryonic connective tissue, the mesenchyma. These cells, to which reference has previously been made, which are known as macrophages, clasmatoocytes, histiocytes and polyblasts (also adventitial cells and pericytes), were originally described by Metchnikoff, who produced evidence that they are concerned with the defense of the organism by ingesting bacteria (phagocytosis) which have made their way into the body and by producing antibodies and forming antitoxins. Attention has been called particularly to the macrophage since the introduction in experimental pathology of supravital staining of animals. Goldmann, after injecting solutions of pyrrol blue into the veins of animals, noted that the dye was taken up by one variety of cells in a special manner, being deposited in the cellular cytoplasm as fine and coarse granules. The red blood cells and the elements of lymphoid and myelogenous origin remained unstained. He observed that these cells are found in all parts of the body, and he identified them with the macrophage of Metchnikoff, the functions of which are those of defense and metabolism. More recent investigations are to the effect that the same variety of cells is also concerned with the defense against and the formation of neoplastic conditions. In brief, at present the mesodermal tissue is looked on as an "organ" performing multiple functions. It is of interest to know whether the lungs, too, are endowed with such an "organ."

In order to investigate the problem I conducted experiments which can be divided into two groups: (1) "sterile" infections (also defined as "model" infections), consisting in the administration to animals of vital dyes and oils respectively, and (2) virtual infections of animals with pathogenic micro-organisms.

### I. "MODEL" INFECTIONS

*Vital Staining.*—Metchnikoff was the first to use dyes in order to observe the behavior of the large mononuclear phagocytes. By placing living sponges in fluid containing carmine or indigo respectively, he noticed that the dyes were taken up by the large mesodermal cells, the macrophages.<sup>2</sup> Bouffard and Ribbert were also using the method of "dyeing" animals in vivo in order to study the reaction of tissues. However, they did not fully appreciate the importance of this technical procedure, and their conclusions were of limited value. Goldmann, whom I have quoted, was the first to emphasize and broaden Metchnikoff's conception that the cells which take up the dye represent a network spoken of as a system or an apparatus spread over the body and possessing common physiologic traits of which the affinity for vital dyes is of particular interest because of the fact that this "earmark" makes them readily identifiable. Thus, by using the method of intravital staining, one is able to recognize the cells of the middle embryonic layer. Aschoff designated this group of cells as the reticulo-endothelial system. I use the term macrophage system, which is rather expressive, which does not prejudge the alleged origin of the cells (reticular and endothelial) and, finally, which renders homage to Metchnikoff, who first observed them.

In examining the tissues of the vitally stained animals, it was observed that while the repeatedly injected dye had stained the abdominal, pelvic and the reproductive organs deeply, it had stained the lungs to a slight degree only. Under the microscope the cells of the histiocytic group were abundantly stained in the bone marrow, the liver, the spleen and the lymph nodes. In the lungs the dye was characteristically stored in the adventitial cells or the pericytes of the larger vessels and in the interalveolar septums. The other pulmonary structures remained either unstained or only faintly tinged with blue.

From similar observations previous investigators concluded that the lungs contain sparse numbers of macrophages.

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2. Metchnikoff was likewise the first to apply the method known as blockade of the macrophage (reticulo-endothelial) system. He noted that under the conditions of his experiments guinea-pigs tolerated intraperitoneal injections of trisulphide of arsenic, the toxic substance being rapidly phagocytosed by the macrophages. But when the injection of arsenic was preceded by that of a solution of carmine the guinea-pigs succumbed. He observed that the large phagocytes "gorged" with the dye remained indifferent to the arsenic which caused the death of the animals.

But when the dye was injected into the lungs via the trachea, the cells lining the air vesicle revealed the following changes: They had enlarged, and their cytoplasm, which had become abundant and "foamy," often contained droplets of pyrrol blue, which was distributed in the manner of histiocytes. Then a number of them seemed to abandon their customary seat on the septum to become wandering alveolar phagocytes, forming the intra-alveolar (inflammatory) exudate.

The endothelium of the capillaries did not show any cytopoietic properties, and the participation of the monocytes from the blood in the process occurred at a later date, probably when the dye had become diffused through the organism in a concentration sufficient to irritate the mesenchyma, leading to proliferation and "release" of local phagocytes.

The origin of the free phagocytic cells found in the air sacs ("dust cells," "heart disease cells") and of the epithelioid cells was the object of investigation by the earliest pathologists. Whereas the partisans of Cohnheim's theory maintained that these cells have reached the lungs from the blood stream by diapedesis (Slavjanski, von Ins), the observers who were opposed to Cohnheim's theory traced the cells to those lining the walls of the alveoli (Ruppert, Arnold) and to those lining the alveolar and bronchial walls. Among modern investigators, Briscoe, Seemann and Westhues, from Aschoff's laboratory, Sewell and Rosin have adopted the views of Ruppert and of Arnold. Permar, a partisan of Mallory's conception, in his studies with vital dyes and also in experimental pneumonia found that "they [the mononuclear phagocytes] arise, in part at least, if not predominantly, from the fixed endothelial cells of the capillary and subcapillary vessels, in the walls of the atria, sacculi alveolares and alveoli pulmonis." Foot, too, published numerous articles in defense of this conception which he has recently discarded for the hematogenous theory. The results of Olch and Ballou's studies on dogs coincided with those described by me. Huguenin and Delarue, who also used dogs in their experiments, have corroborated my observations on small laboratory animals.

*Intratracheal Injection of Oils.*—When the lungs were removed and examined about one hour after the intratracheal injection of oils (iodized poppy seed oil 40 per cent, cod liver oil and liquid purified petroleum benzine), the reaction they produced was essentially that of the cells lining the wall of the air sacs. These cells revealed a cytoplasm which contained vacuoles of various size and a hyperchromatic round or oval nucleus (fig. 1). They protruded above the surface of the alveolar wall in a budlike manner, and a number of them lay free in the alveoli and contained phagocytosed fat in their cytoplasm. Within from twelve to twenty-four hours following the injection, the cells of the alveolar wall showed a marked increase in number and also in size; they appeared as large foam cells, thus leading to thickening of the alveolar septums. At the end of the second and third days the cellular proliferation caused atelectatic areas. In places the greatly enlarged cells lining the septum and those accumulated in the pulmonary alveoli produced continuous cellular masses resembling xanthomatous nodules or agglomerations of proliferated cells like those seen in Gaucher's disease (fig. 2).



Neither the endothelium of the capillaries nor the bronchial epithelium participated in the reaction. The afflux of phagocytic cells from the blood stream was insignificant, and their rôle in the formation of the intra-alveolar exudate was slight.

Waldeyer was probably the first to note that "the embryonic cells of the connective tissue," or *Plasmazellen*, take up fat avidly; he spoke

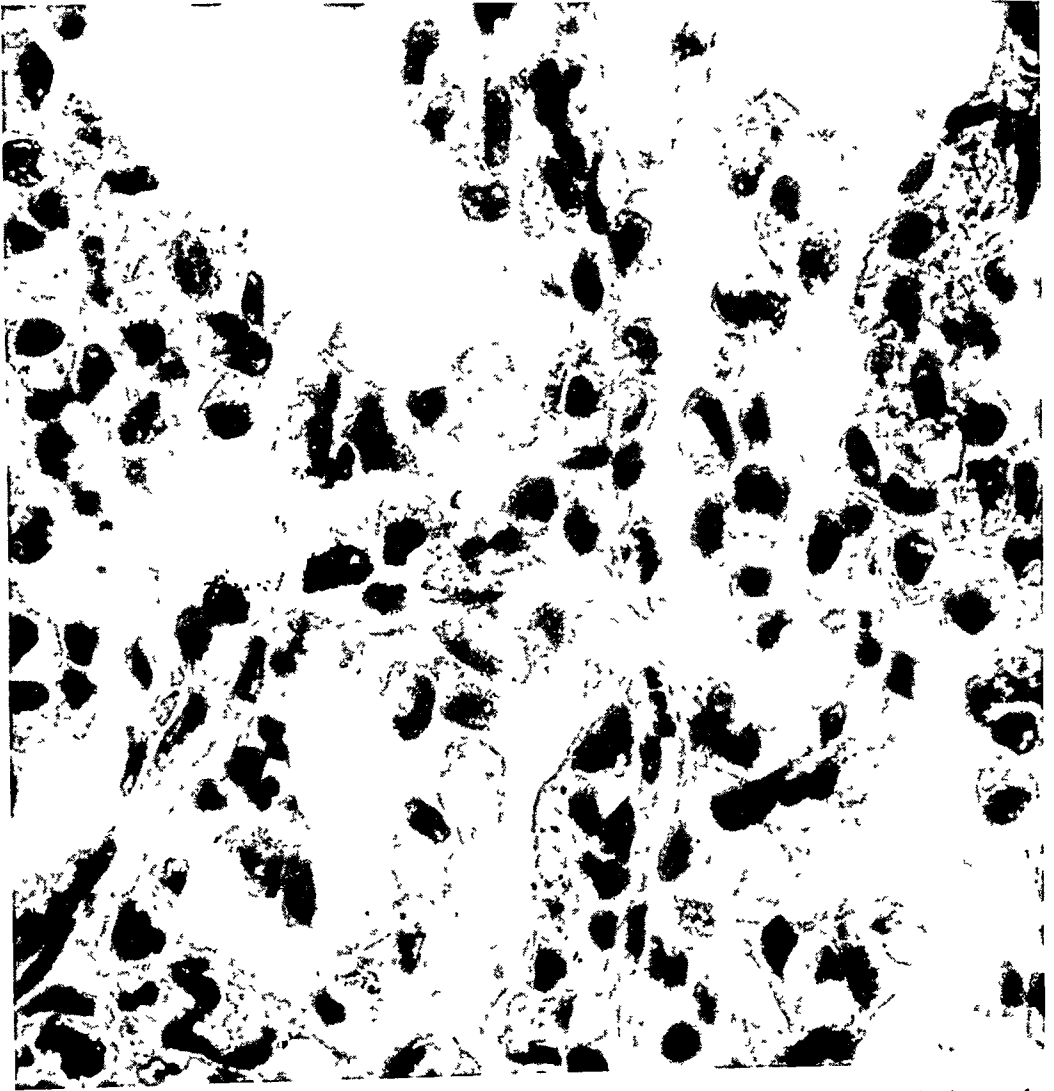


Fig 1.—Fixed and wandering cells of the air sac, showing their similarity and their close resemblance to macrophages (histiocytes).

of these cells as "grosse rund Zellen welche besonders gern Fett aufnehmen" ("large round cells which absorb fat with avidity"). François noticed that these cells "transform" themselves into fat cells which at one time was considered to be a pathologic condition, but which subsequent observations have revealed to be a physiologic metabolic change. Schultze observed the rôle of these cells in diabetic lipemia. In per-

forming a necropsy on a patient with diabetes and lipemia, he found an enlarged spleen which on histologic examination showed proliferated and hypertrophied reticular cells (fig. 3). He expressed the opinion that the spleen most likely plays a rôle in the metabolism of lipoids and suggested that this property resides in the reticular cells (splenic macrophages, or splenocytes). This was subsequently corroborated by

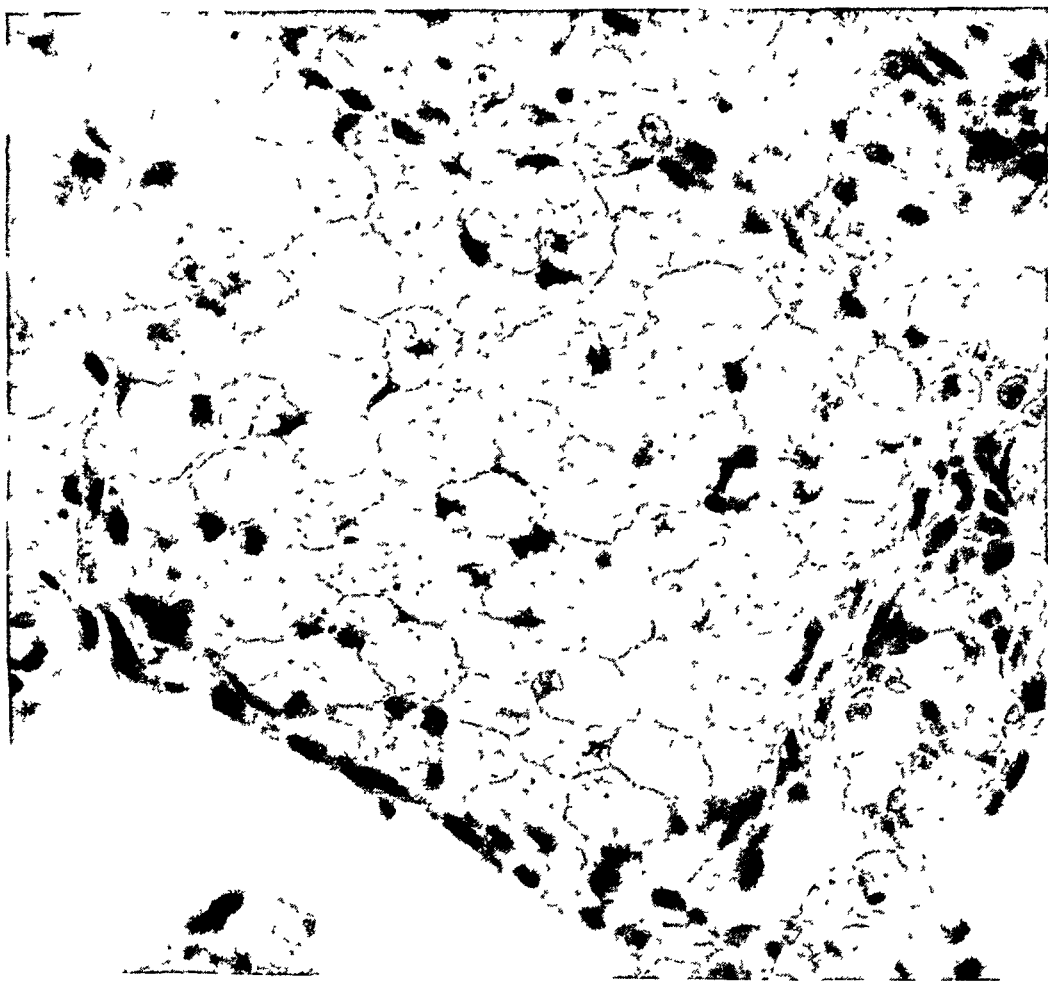


Fig. 2.—An intra-alveolar xanthomatous nodule made up of proliferated cells of the alveolar wall.

many observers, who also found that similar processes occur in nephrosis, in obstructive jaundice and in other pathologic conditions.

More recent studies on Gaucher's and on Niemann-Pick's disease and also on the disease known as the Schuller-Christian syndrome (xanthomatosis) have likewise revealed that the lipoids whose metabolism is disturbed are phagocytosed essentially by the macrophages (Rowland). Similarly, experimental studies by Anitchkow and by Zinserling revealed

that small laboratory animals which were fed an excess of cholesterol emulsified in sunflower oil showed in the intima of the vessels large cells studded with lipoids, which were readily identified as macrophages. Thus, when rabbits are fed an excess of pure cholesterol or yolk of egg, the lipoids are "stored" in the spleen and the bone marrow, which, on microscopic examination, reveal that the material is deposited exclusively in the reticular cells and in the cells which line the sinuses of the spleen. As the result of the excessive feeding of cholesterol these

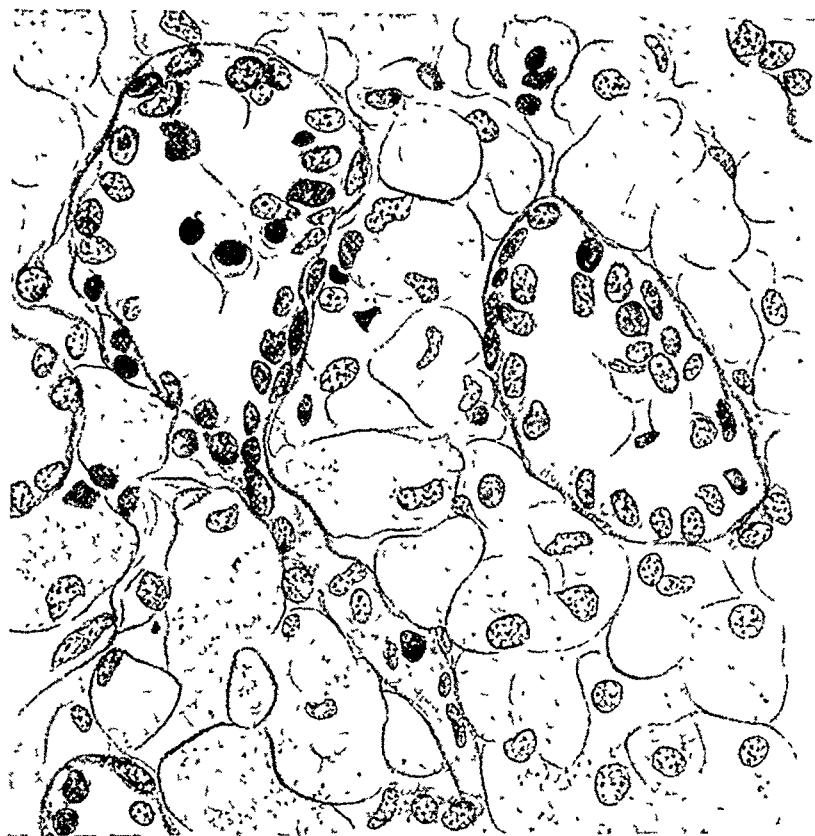


Fig 3.—Structure of the spleen in diabetic lipemia (Schultze). The dilated sinuses lined with proliferated and hypertrophic macrophages were formerly described by investigators as "cystic structures lined with epithelial cells."

cells enlarge and proliferate, forming wide agglomerations in the splenic pulp and also in the sinuses. In the bone marrow, the orally introduced fatty substances lead to cellular changes similar to those found in the spleen. Anitchkow noticed that cholesterol artificially increased in the organism is deposited largely as anisotropic fat in the macrophages of the connective tissue, with the formation of typical xanthoma cells.

Oils introduced into the respiratory portion of the lungs via the trachea cause a reaction in which the alveolar "epithelial" cells (also the

peribronchial and perivascular macrophages) are primarily involved. The behavior of these cells is a counterpart of that of the macrophages in the spleen, liver and bone marrow. Seemann, after injecting fine emulsions of cholesterol into the circulation of laboratory animals, observed that in the lungs the lipoid substance was "fixed" by the cells lining the alveoli, being deposited and "stored" in their cytoplasm, as is done by the macrophages elsewhere in the body.

These observations are of interest in connection with the hypothesis expressed by numerous writers that the lungs play a rôle in the metabolism of fat. Roger compared the pulmonary action on fatty substances with the action of the liver on carbohydrates. This comparison was suggested by the fact that absorbed albumins and carbohydrates pass directly into the liver, while fatty substances are transported by way of the lymphatics and the thoracic duct to the right side of the heart and to the lungs. Extensive studies by Derman and Leites were to the same effect. Markowitz and Mann, in a more recent report, stated that the results of their experiments "have not furnished evidence for the supposition that the lung plays an especial rôle in the metabolism of fat." However, they, too, found that in one experiment, in which a highly emulsified preparation of fat was injected intravenously, there was a huge increase of fat in the lung and a definite diminution of fat in the blood from the femoral artery as compared with blood removed from the right ventricle.

Apparently the problem is complex, and further experiments will be required. As Ranvier expressed it: "Quoi de plus aisé que de montrer aujourd'hui la présence de glycogène dans le foie. Cependant, Claude Bernard, qui était un homme de génie, n'y est arrivé qu'après bien des années de recherches et par une série de tâtonnement." ("Nothing is easier today than to demonstrate the presence of glycogen in the liver; yet Claude Bernard who was a genius, succeeded in doing so only after numerous attempts and years of research.")<sup>3</sup>

## II. VIRTUAL INFECTIONS

*Infection of Animals with the Anthrax Bacillus Via the Trachea.*—The "model infections" have shown that the lungs possess a cellular "apparatus" which to all appearances is a counterpart of the macrophage apparatus found throughout the body. It also became apparent that the cells of which it is made up are chiefly those which line the air sacs and which are commonly designated as alveolar epithelium. The

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3. I have observed in man and in lower animals that the alveolar epithelium plays a rôle in the local (extrahepatic) formation of bile. By inducing hematomas in the lungs of animals, bilirubinemia can be demonstrated.

experiments were undertaken in order to study the behavior of these cells in instances of virtual infection with pathogenic micro-organisms.

The experiments with the anthrax bacillus can be divided into two series: (1) a series in which the rabbits receiving the injection of the gram-positive bacillus by way of the trachea were left alive for observation, and (2) a series in which the animals were killed at intervals of from five minutes to three weeks after the intratracheal infection. The experiments showed that manifold fatal doses of the anthrax bacillus can be introduced into the lungs of rabbits with impunity when the micro-organism is brought directly in contact with the pulmonary tissue, provided that the extrapulmonary tissues are spared contamination. The micro-organisms so inoculated are retained by the lungs, where they are destroyed within a few hours after injection by phagocytosis, in which the cells found in groups along the wall of the air sacs (alveolar epithelium) play the outstanding rôle.

Although the anthrax bacillus belongs to the micro-organisms that have been discovered earlier (Davaine, Pasteur), the mechanism of death caused by this *batonnet* remains to be explained. As no observer has succeeded in isolating a toxin from this micro-organism, it was presumed that it causes death not by endotoxins or exotoxins but by virtue of its tremendous proliferation within the circulation, which results in occlusion of vessels culminating in asphyxia. However, as has been stated, recent investigations have revealed that when the bacillus is injected into the blood of animals it disappears rapidly from the circulation; it is lodged in the internal organs and reappears in the peripheral circulation shortly before the animals' demise (Besredka). Septicemia is, then, a terminal stage, simply heralding the fact that the host has lost the fight and that the offender is released. It is also remarkable that in this infection, probably more than in any other, local trauma plays an outstanding rôle in the spread of the disease. Finally, it was noted by observers both formerly and recently that the micro-organism is harmless to laboratory animals when injected into the lung with the precautions used in my experiments (Morse, Hildebrandt, Grammatchikoff, Snel, Besredka, Balteano, Brocq-Rousseau and Urbain, Combiesco, Gracia).

Besredka was the first to stress the fact that in the lower animal the skin is the sole organ that is susceptible to the anthrax bacillus, whereas other organs or structures resist infection. Thus, he stated that "chez le cobaye il n'existe qu'un organ pour lequel la bactériodie ressent une réelle affinité, organ dans lequel elle peut s'implanter, au sein duquel elle peut croître et se multiplier: cet organ est la peau." (In the guinea-pig only one organ exists for which the anthrax bacillus shows a virtual affinity and in which it is able to implant itself, grow and multiply; that organ is the skin.") "La bactériodie," he stated further, "que pénètre ailleurs que dans la peau ou le tissu sous-cutané,

passé évidemment inaperçue de l'animal: aussitôt arrivée dans le péritoine ou dans le poumon elle y est aussitôt phagocytée et digérée." ("The anthrax bacillus which enters by other means than the skin or the subcutaneous tissue remains unnoticed by the animal; in the peritoneum or in the lungs it is phagocytosed and digested the moment it has invaded these organs.")

The experiments with the anthrax bacillus belong to the series of postwar studies which served Besredka as a point of departure for his conception of local immunity. His work on local immunity to pathogenic micro-organisms has been corroborated by a number of observers, who also were concerned with the mechanism of this phenomenon. For details on this phase of the problem the studies by Gay and his associates and by Cannon and Pacheco are of interest. Gay, who made extensive studies on the subject, produced evidence that "the clasmato-cytes or 'tissue macrophages' are in part, if not entirely, responsible for the natural resistance of rabbits to experimental streptococcus infection." He also made the important observation that the "native" resistance varies from structure to structure, depending on the abundance of macrophages with which the region is provided. Thus, the peritoneal cavity is at least a thousand times more resistant to streptococcic infection than the pleural cavity. It is also remarkable that the macrophages are mobilized from the adjacent connective tissue and not from the circulating blood. Cannon and Pacheco found that the local immunity of the skin resulting from previous immunization by intracutaneous injections of staphylococcus vaccine is predominantly cellular in type, with the tissue macrophages playing the dominant rôle, owing to increased numbers and also, probably to increased metabolic activity.

From the foregoing observations it is apparent that the macrophage is the element *par excellence* which is concerned in the process of local immunity of tissues to invaders. "Les propriétés humorales," wrote Metchnikoff, "ne représentent qu'une certaine fraction dans l'ensemble des phénomènes de l'immunité, cette dernière étant dominée par des propriétés cellulaires, qui apparaissent dans la presque totalité de cas d'immunité naturelle ou acquise." ("In the complete phenomenon of immunity, the humoral properties represent but a fraction, the phenomenon being dominated by the properties of the cells which are conspicuous in nearly all cases of natural and acquired immunity.") This statement, made more than thirty years ago, has been recently confirmed by observers who used modern methods of investigation, such as tissue cultures.

Apparently, in the experiments with the intratracheal injection of the anthrax bacillus the same defensive mechanism intervened. It is, however, remarkable that the outstanding rôle of the scavenger which

disposes of the pathogenic *batonnet* was played by the cells lining the wall of the air sacs, which from time immemorial have been regarded not as macrophages but as epithelial cells. This observation was particularly evident from the infection of animals with the tubercle bacillus which is described in the following section.

*Infection of Rabbits with Tubercle Bacilli by Way of the Trachea.*—In initiating a study on experimental tuberculosis, the respiratory route of infection was chosen in order to imitate the technic of the "model" infections with dyes and oils. A survey of the literature has revealed a few incomplete reports on this method of infection with Koch's bacillus. Indeed, the results reported by previous investigators were usually based on hematogenous methods of infection whereby a condition of acute sepsis with Koch's bacillus was induced in animals. It is accepted that in man tuberculosis is as a rule initiated by inhalation of the acid-fast bacilli, whereas a primary infection by way of the blood stream is very rare. In the lower animals the two forms of infection respectively cause diseases the natural histories of which vary to a great extent, the aerogenous being probably closer to the form observed in man. The experiments were conducted on full-grown rabbits.

Microscopic examination of tissues revealed that when the tubercle bacilli reached the air sacs the reaction was instantaneous: within a few minutes there occurred proliferation, as well as morphologic changes of cells lying in and on the septums. The latter cells which are normally barely visible, giving to the air sac a quasi-naked appearance, revealed characteristics that are proper to fixed or wandering macrophages (monocytes, clasmatocytes). A number of these cells lay free in the alveolar lumen, showing phagocytosis of the tubercle bacilli. Tubercle bacilli were also found in the cytoplasm of some of the enlarged sessile cells. Within the first hour after the infection primitive tubercles made up of the cells lining the air sacs (respiratory epithelial transformed into epithelioid cells) were seen scattered over the sections. At a later period outlines of giant cells could be made out, resulting from agglutination of individual cells and also from the nuclear division of cells.

As a rule, newly formed cells proliferated inside the lumen of the alveolus, (fig. 4) forming a microscopic pneumonic focus or a "parenchymatous alveolitis" (*alvéolite macrophagique* of the French writers). This process ordinarily involved a group of several alveoli, the septums of which could be outlined early in the disease.

In addition to the prompt morphologic changes and the rapid proliferation, the sessile and free cells displayed instantaneous phagocytosis of the acid-fast bacilli. Not only did the morbid process appear to advance by proliferation of its own elements, but at the borders it "converted" the adjacent cells. Here, too, the cells, while still resting on the septums, had taken on new morphologic aspects closely resembling those of the large monocytes. In proximity to the primitive tubercle these cells could be seen to "stream" singly or in groups to join and enforce the already formed miliary nodule. Thus, when the agglomerated tubercle had reached considerable dimensions and its center had begun to degenerate

(caseation), its periphery was made up of new elements that resulted not only from proliferation of its own cells but also from the mobilization of those resting in the adjacent area.

The endothelium of the capillaries of the septums showed no particular changes. The lumens of the septal capillaries contained a moderate number of red cells. The pulmonary lymphocytes did not seem to play a rôle in the provision of the first "waves" of macrophages.

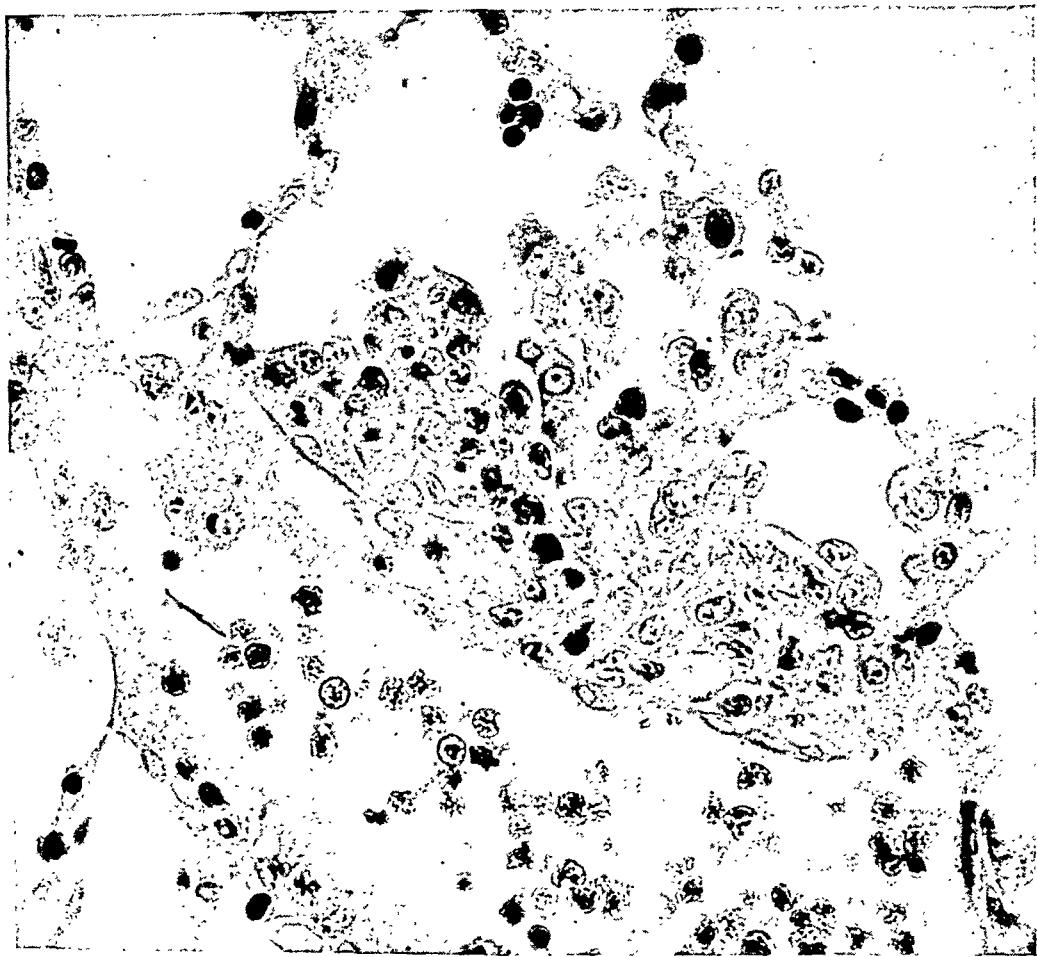


Fig. 4.—Section of the lung of a rabbit infected with tubercle bacilli, showing the early stage of parenchymatous alveolitis.

Virchow regarded the tubercle in tuberculosis as a heterologous growth (neoplasia), taking its origin from connective tissue analogous to that found in neoplastic diseases. However, the opinion was prevalent among his contemporaries that the miliary tubercle is formed from all kinds of fixed tissues: the epithelium of the bronchi and the respiratory tract, the endothelium lining the blood vessels, the polygonal hepatic cells and the epithelium lining the bile ducts, renal epithelium, muscle fibers and the like (Thaon, Grancher, Strauss, Baum-



garten and others). As has already been stated, although the cell dominated the teaching of pathology, cellular specificity was but vaguely understood until the advent of Metchnikoff.

According to Metchnikoff, "the tubercle is made up of a collection of phagocytes of mesoblastic origin, which flock toward the areas where the bacilli are located and engulf them." "In the lungs," he stated elsewhere, "the tubercle is formed at the expense of the endothelial cells of the blood vessels with assistance of leukocytes." Borrel, who, after Metchnikoff, made a comprehensive experimental study on tuberculosis, wrote as follows: "Le système lymphatique est la gangue ou se développent les tubercules, et non le tissu conjonctif. La cellule tuberculeuse est toujours une cellule lymphatique." ("The lymphatic system and not the connective tissue is the soil where the tubercles develop. The tuberculous cell is always a lymphatic cell.") Calmette fully subscribed to Borrel's findings by stating that "dans les poumons comme dans les séreuses, l'élection des tubercules autour des vaisseaux est due à cette particularité qu'ils se développent presque exclusivement dans le système lymphatique. Ce dernier est la gangue ou se constituent les tubercules, et non le tissu conjonctif comme le prétendait Virchow." ("In the lungs as in the serous membrane the elective seat of the tubercles around the vessels is due to the fact that they develop almost exclusively in the lymphatic system. The latter is the terrain where the tubercles are formed, and not the connective tissue, as was claimed by Virchow.") Baldwin, Petroff and Gardner, in a recent monograph, emphasized that "without exception tubercle is formed in the depth of the tissues, by preference in lymphoid masses, but where the latter do not occur, in and about lymphatic vessels; never does the tubercle start upon an epithelial surface. Applying these propositions to the case of the lung, tubercle could not develop upon the surface of the respiratory epithelium, nor would it be expected within the wall of the air spaces which are devoid of lymphatic vessels." Finally, Krause stated that "added years of uninterrupted observation of the primitive tubercle have served only to strengthen the idea of at least one worker, that this parent cell exists *in situ*, and in a thoroughly topographical sense, may be thought of and designated as a fixed tissue cell; that it is not exuded from the blood or lymph, or called as by *taxi*, from a distance in response to tubercle bacilli."

Pathologists today share fully Metchnikoff's views as to the mesodermal origin of the epithelioid cells of which the tubercle of the lung is made up. They are, however, in disaccord as to their source. As in the case of the origin of inflammatory exudate elsewhere in the body, investigators have adhered either to the theory of endothelial leukocytes of Mallory, or to that of the hematogenous origin of the epi-

thelioid cells; to the view that they arise in local connective tissue or to the view that they have a double origin—hematogenous and local.

The endothelial origin of the pulmonary epithelioid cells have been combated by Aschoff and by Maximow. I, too, could find no evidence of the participation of the endothelium of the pulmonary capillaries in the provision of the epithelioid cells. This conception has been recently abandoned by the "younger" pathologists (Foot, Gardner) and apparently by Mallory himself. Thus, in a study made in Mallory's laboratory, Nye and Parker stated as follows: "In the past, many writers have claimed that the capillary endothelium in the lungs possesses great phagocytic powers, but few at present hold this view. There is no evidence that the monocytes in the lungs are derived from the *circulation* or from the *capillary endothelium* [italics mine]." They believed that the source of the pulmonary phagocyte lies in the lungs themselves and not in the extrapulmonary tissues, but they were not certain as to its precise location. Gardner and Smith, too, found that migration (into the lungs) of cells from the blood stream is eliminated because alveolar phagocytes accumulate after the death of the animal, when the blood is no longer circulating.

Apparently Foot is the sole author who recently expressed the opinion that the alveolar phagocytes are of hematogenous origin. In view of the fact that he attributed the same conception to Metchnikoff, it is pertinent to look into this investigator's statements.

In his first article on phagocytosis of Koch's bacillus, published in 1888, Metchnikoff wrote: "Die Lungen-Tuberkelbildung bei Kaninchen (nach Injektion der Kulturen in die Ohrvene) erfolgt in *wenigen Tagen* wobei man starke Anhäufungen von *fertigen* Epithelioidzellen, sowie von Lymphozyten beobachten kann [italics mine]." ("The formation of tubercles in the lungs of rabbits [after the injection of cultures in the vein of the ear] follows within a *few days*, whereby one observes massive accumulations of *fully developed* epithelioid cells and lymphocytes.") The source of the "*fertige Epithelioidzellen*" in the lungs was not specified. In his "Leçons sur la pathologie comparée de l'inflammation" he stated: "Après les leucocytes, ce sont les vaisseaux et leurs endothéliums qui jouent le rôle le plus important dans l'inflammation . . . les éléments endothéliaux ont conservé encore plusieurs traits de mobilité, attestant leur origin. . . . Le tubercule pulmonaire se forme aux dépens des cellules endothéliales des vaisseaux sanguins avec le concours des leucocytes." ("After the leukocytes, the vessels and the endothelium play the most important rôle in inflammation . . . the endothelial elements in accord with their origin have preserved many features of motility. . . . The pulmonary tubercle is formed at the expense of the endothelial cells of the blood vessels

with the assistance of leukocytes.") The source of the latter was described by him in his monograph "L'immunité dans les maladies infectieuses," in which he wrote: "For a long time the large 'dust cells' of the respiratory channels were looked on as being epithelial cells that were capable of taking up carbon particles, micro-organisms and other foreign bodies. In reality these elements are none other than white corpuscles that have immigrated into the alveoli and bronchi." In the spleen and the lymph nodes the tubercle, according to Metchnikoff, results from a collection of the large mononuclear phagocytes of these organs and not from outside sources.

In connection with Metchnikoff's opinion, it is of interest to cite that of his contemporary, Borrel, on the same subject. Borrel, who used the hematogenous method of infection with the tubercle bacilli, found, as has already been stated, that the cells in the tuberculous lesion are always of "lymphatic" origin. In another section of his article dealing with the alveolar response he wrote as follows: "Dans les alvéoles il existe une catégorie de cellules qui jouent le même rôle que les grands leucocytes mononucléaires dans les vaisseaux, ce sont les cellules à poussidères, les *Staubzellen* des auteurs allemands." ("There exists in the alveoli a category of cells which play a rôle equivalent to that of the large mononuclear leukocytes found in the blood vessels; these are the dust cells, the *Staubzellen* of the German authors.") He did not observe them in the blood-borne infection with Koch's bacillus, but he mentioned casually that, as a result of the "inhalations de grandes quantités de bacilles tuberculeux, on constate alors au microscope, des premiers jours, l'envahissement des alvéoles pulmonaires par une énorme quantité de cellules à poussières; la plupart de ces cellules contiennent des bacilles qui sont comme noyés dans un épanchement non moins considérable de leucocytes polynucléaires." ("After the inhalation of large quantities of tubercle bacilli, microscopic observation shows from the first the invasion of the pulmonary alveoli by an enormous quantity of dust cells. Most of these cells contain bacilli, which appear as if drowned in a not less considerable exudate of polymorphonuclear leukocytes.") Apparently Borrel discriminated between two strains of cells: the immigrated large mononuclear leukocyte and the "dust cell." He suspected that the latter was indigenous in the lung and that it displayed its activity in massive aerogenous infection.

It may be seen that the theory of the essentially hematogenous origin of the dust cells and of the pulmonary epithelioid cells forming the tubercle was erroneously attributed to Metchnikoff.

Of particular interest in this respect are the experiments of Lang and of Timofejevski and of Benevolenskja, who made cultures of lung tissue inoculated with tubercle bacilli. In experiments, in which circu-

lation was excluded. these investigators showed that the lungs and no other organs represented the source for the alveolar phagocytes.

The findings of Borrel, of Calmette and of Baldwin and his associates that "the tubercle is formed . . . by preference in lymphoid masses" resulted probably from observation on hematogenous infection with the tubercle bacilli. The vascular supply of the lymphoid tissue found within the lung of the rabbit is derived from the pulmonary artery (Miller). "Therefore," wrote Krause, "in the rabbit, bacteria that have gained the general venous system will, in part at least, be conveyed to the lymphoid tissue directly." However, even here, the tubercle, i. e., the epithelioid cells, originate not from the lymphocytes, but from the clear central zone of the reticular cells, which are none other than macrophages.

Thus the notion held by authorities that the blood stream and the endothelium are the essential contributors to the dust cells and the epithelioid cells forming the tubercles in the lung is today abandoned.

#### COMMENT

From my observations it became apparent: (1) that the large mononuclear cells found in the pulmonary alveoli in inflammatory and congestive processes are macrophages; (2) that they originate from the cells found along the wall of the air vesicles known as respiratory epithelium. This observation is shared by the majority of modern pathologists (Aschoff, Bloom, Seemann, Marchand, Maximow, Carlton, Cappell, Lang, Pagel, Policard, Huguenin, Tchistovitch, Chiodi). The point of dispute is the nature of these cells, which are regarded by one group of observers (Aschoff and his school) as endodermal, and by others (Maximow, Lang, Bloom, Policard and myself) as mesodermal.

Seemann, as the result of experiments carried out in Aschoff's laboratory, related findings closely approaching my own, maintaining, none the less, that the cells lining the air sacs are of endodermal origin. He based his opinion on "classic" embryologic data and on the results obtained by staining tissues from lungs with silver nitrate. He did not discuss in detail the macrophagic traits of these cells, but dismissed the subject by saying that "the outstanding activity of the 'alveolar epithelium' should not surprise us if we remember that *occasionally* [italics mine] the liver, kidney, adrenal and testicular cells are strongly phagocytic." This statement cannot be relied on, since these cells are passive (facultative) phagocytes, whereas the alveolar "epithelium" is actively (obligatorily) phagocytic. This is of importance in connection with the statement made by Aschoff: "It is known that occasionally every possible cell will 'swallow' coarse foreign elements; i. e., it will react as a macrophage. . . . There is hardly a fixed cell in the

organism that is not in a position, under certain circumstances, to swallow and to digest other cells, foreign bodies and parasites. Are they thereby functionally identical? Not to the slightest degree. There is concerned here one of the functions proper to every cell, that is, of digestion. One must, therefore, also look for other characteristics. In the cell with which we are concerned (the reticulo-endothelial) phagocytosis is only one property which is particularly marked. The intensity and the amount of phagocytosis are here the deciding factors." Then, too, the "phagocytic" epithelial cells of the viscera mentioned by Seemann do not show proliferation as a result of "irritation" and do not transform themselves into epithelioid cells under the influence of the tubercle bacillus. They *occasionally* engulf foreign elements while still attached to their basal membranes, but are invariably found to be dead when detached from their customary seat, whereas the alveolar "epithelial" cells display ameboid phagocytic activities in space. Staining with silver nitrate, the method applied by Seemann to prove his thesis, is regarded by most observers to lead to ambiguous pictures. Finally, the nature of the structure of the fetal lung, which serves as an argument in support of the epithelial nature of the epithelial cells, is far from being definitely determined. Today, as in the time of Virchow, "the doctrine of embryologic leaflets is not yet clearly elucidated in all its details and . . . every new investigation along this line brings some modifications" (Virchow).

Embryology teaches that the air sacs of the embryo are lined with a single uninterrupted layer of cuboidal epithelial cells. It is assumed that as a result of the first respiratory effect some of these cells become abruptly flattened and some lose their nuclei altogether, leading to the formation of the anucleated platelike cells of the functioning alveolus. The abrupt flattening of the cuboidal cells following the first respiratory movement and the function of the anucleated cells have, however, never been plausibly explained. In fact, recent observers deny their existence. "Ce sont des 'êtres de raison' acceptés par habitude et par respect d'un schema traditionnel," according to Policard. ("These are 'creatures of reason' accepted by habit and out of respect for tradition.")

With regard to the abrupt flattening of the "respiratory epithelium," Fauré-Fremiet and Dragoiu found that in the sheep the transformation is not abrupt but occurs gradually, beginning in the second half of embryonic life, at which time free spaces appear between the alveolar cells. At this juncture the nucleated cells take on a new aspect and acquire new potentialities. These investigators observed, for instance, that the cells contain a chemical substance closely akin to glutathione described by Hopkins. "It is certain," stated Huguenin, Foulon and Delarue, as a result of experiments performed in Roussy's laboratory,

"that the cells which at one time were morphologically and physiologically epithelial are replaced by elements which are the future granular (foam) cells of the adult lung." Policard and Chiodi believed that the cuboidal cells lining the fetal alveoli completely disappear, thus "denuding" the respiratory sac, which is subsequently partly "relined" by immigrated mesenchymal cells. In this connection it may be of interest to note that during embryonic life the esophagus, too, changes its lining on five occasions. It also was observed that even in postembryonic life processes of involution of organs or structures occur. Recent studies by Smith and Bennet, made in Wolbach's laboratories, revealed that in postembryonic life the size and number of the alveolar cells in the rat vary with age.

Bloom reproduced in Maximow's textbook of histology two photographs of the lungs of an embryo guinea-pig near term to show the change which takes place in the embryonic lung when the air spaces become distended with fixing fluid. "It is clear," wrote Bloom, "that at this stage, even before injection of the fixative, there is no longer a cuboidal lining of the alveoli."

No systematic studies on the embryology of the lung were made by me. Casual observations with the use of the technic of insufflation of the fetal lung within physiologic limits (a method ordinarily used by me in experiments on postembryonic lungs) have revealed pictures hitherto unobserved. Thus, figure 5 *A* is a photomicrograph of a section of a lung removed from an embryo guinea-pig measuring 2.5 cm. in length. The picture illustrates the peculiar alveolar structure of the fetal lung in the early stages. In figure 5 *B* a section from the lung of an embryo guinea-pig 6.5 cm. long (near term) is shown. It may be seen that at this period the classic "adenomatous" structure no longer exists.

The observation on the imperfect lining of the air sacs was known to earlier observers, such as Zenker, Villemin, Todd and others. Claude Bernard, in studying the absorbing power of the mucous membrane of different organs, found that toxins and poisons are absorbed by way of the alveolar surface as rapidly as by way of the blood stream. He noticed that curare placed on the mucosa of the bronchi caused no harm to the animal, but that when this alkaloid was "pushed" into the alveoli it killed the animal as rapidly as if it had been injected into the circulation. He attributed this phenomenon to the lack of an "epithelium protecteur à la surface des vesicules pulmonaires." In a recent study Policard stated: "The respiratory surface of the lungs ought to be compared to an open wound."

In some pathologic conditions of the lungs structures resembling acini lined by cuboidal cells occur. These were interpreted as respiratory

alveoli lined by cuboidal cells that have reclaimed their ancestral embryonic form. I have observed that the glandular structures present in sclerosed lungs are often no other than newly formed alveolar structures

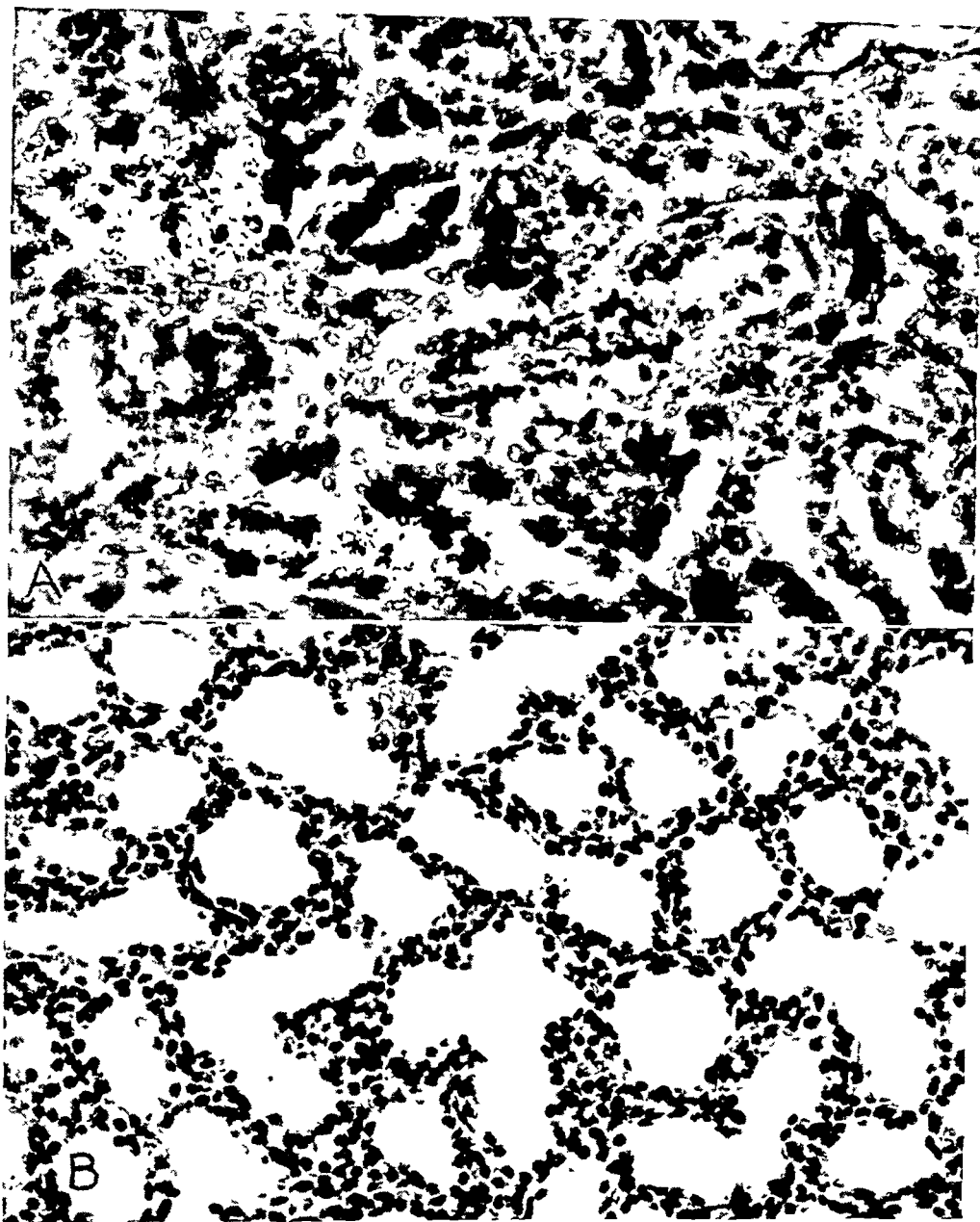


Fig. 5.—*A*, section of the lung of an embryo guinea-pig 2.5 cm. long. The glandlike structure should be noted. *B*, section of the lung of an embryo guinea-pig 6.5 cm. long, i. e., near term. The “glands” seen in the earlier stages of development no longer exist.

lined by cells of bronchiolar origin. Analogous pictures are observed in atrophic (Laennec's) cirrhosis of the liver, in which there also occurs a new formation of bile ducts within the rings of connective tissue

encircling the distorted hepatic lobules. In diffuse sclerogenic disorders of the lungs the respiratory alveoli, too, appear like glandular structures lined by an uninterrupted layer of cuboidal cells, an appearance which



Fig. 6.—Lining of a pulmonary alveolus in a diffusely fibrosed human lung. The similarity of the sessile and the free cells, which have all the earmarks of macrophages, should be noted. Two free cells ("heart disease cells") contain blood pigment. Reduced from  $\times 1,100$ .

has often been cited as evidence of the epithelial nature of the alveolar cells. However, a study of numerous lungs with similar pictures has convinced me that here, too, the cells of the alveolar wall behave in ways similar to that of macrophages and that their morphology is often



that of large mononuclear phagocytes, as is seen in figure 6. Similar pictures are observed in the sinuses of the spleen in diabetic lipemia, in Gaucher's disease, in Niemann-Pick's disease and also in cirrhotic conditions of the spleen. The splenic sinuses in these diseases have been often referred to as "cystic structures lined with cuboidal epithelium" (fig. 3).

Experimental and clinical observations point to the conclusion that the so-called epithelial cells which are found singly and in groups along



Fig. 7.—Cut surface of the lungs of a rabbit infected with bovine tubercle bacilli by way of the blood stream, showing diffuse miliary tuberculosis. The animal lived six weeks after the infection.

the septums of the pulmonary alveoli are probably of mesenchymal origin; that is to say, they are "resting" macrophages and accordingly produce cells of their kind, i. e., the "wandering" macrophages of the air sac. This hypothesis is also supported by the examination of pulmonary tissue inoculated with tubercle bacilli in vitro. Thus, Lang found that the alveolar phagocytes originate from the alveolar "epithelium," which he regarded as being polyblasts or macrophages. He emphasized that they are a counterpart of those present inside the sep-

tums. He likewise observed that the phagocytic cells which are found around the pulmonary vessels and bronchi are similar to the cells lying in and on the septums.

The behavior of the lungs in instances of infection by way of the trachea reveals that the respiratory portion of these organs possesses an exquisitely efficient defensive structure. It is remarkable that the



Fig. 8.—Cut surface of the lungs of a rabbit infected with bovine tubercle bacilli via the trachea. The lungs are markedly enlarged and show nearly complete caseation. The heart is pushed upward, and the upper lobe of the left lung is reduced in size as a result of compression. There is an adhesive fibrous pleuritis leading to obliteration of both pleural cavities. The animal lived four times longer than its fellow, the lungs of which are shown in figure 7. Both animals were of the same weight and age at the time when they were infected with the same amount of tubercle bacilli.

thin and "half naked" alveolar wall which has no more resistance than a "spider's web" in regard to fluids, is a virtual fortress when bacteria have penetrated into the respiratory portion of the lungs.

The difficulties encountered in inducing a pulmonary disease in animals infected via the trachea have been baffling observers since the advent of bacteriology. I have observed that a dose of pneumococci that kills a rabbit in about eighteen hours, when injected into the blood stream, causes the death of another rabbit infected by way of the trachea in about four days. On the other hand, a dose of this micro-organism sufficient to cause the death of a hematogenously infected animal within from three to four days is harmless to an animal infected via the respiratory tract. The micro-organism is promptly phagocytosed and destroyed by the alveolar "epithelial" cells. Likewise, in the resolution of lobar (fibrinous) pneumonia, the alveolar "epithelial" cells and not the granulocytes play the rôle of scavengers, performing this function by phagocytosis and also by secreting lytic substances. The granulocytes flood the lung in the early stages of the disease, being supplanted later by alveolar ("epithelial") phagocytes.

I have mentioned the differences existing between the hematogenous and the aerogenous infection of rabbits with the tubercle bacilli (figs. 6 and 7). Thus, the duration of the disease in animals infected with Koch's bacillus via the trachea is about four times longer than in those infected by way of the blood stream. The disease is disseminated throughout the body at a much later period in aerogenous infection, possibly in the terminal period, and in some instances it is confined for life to the lungs.

The efficacy of the pulmonary filter in regard to pathogenic bacteria was stressed by Besredka in the following terms: "Tant qu'il est intact, le filtre pulmonaire est imperméable à la façon d'une bougie à pores serrées; mais il suffit la lésion la plus minime pour que sa porosité augmente au point de rendre le filtre inopérant." ("So long as the pulmonary filter is intact it is as impermeable as a candle with tight pores. But the slightest lesion is sufficient to increase its porosity to such a degree as to render the filter ineffective.")

#### SUMMARY

Since the time of Magendie and Claude Bernard it has become known that of all mucous membranes that of the lungs possesses the best absorbing power. Thus, alkaloids or dyes injected into the lungs via the trachea are detected in the general circulation and in the urine within a few minutes after the injection.

On the other hand, since the advent of the bacteriologic era it became obvious that fine emulsions of pathogenic bacteria which are fatal to animals by intraperitoneal, subcutaneous or hematogenous infection are harmless when the respiratory portion of the lungs is chosen as an avenue for the infection.

The experiments reported in this review were undertaken in order to study the mechanism whereby the lungs get rid of the pathogenic invaders, thus maintaining their sterility and serving as a barrier against a systemic dissemination of the disease.

The studies have revealed that in the lungs, as elsewhere in the organism, the protective function lies essentially in the macrophages, which dispose of the invader by means of phagocytosis.

It was further demonstrated that in the early phases of pulmonary infection these cells are not imported from outside sources but are abundantly supplied by the lungs themselves.

It was shown, moreover, that most of the wandering macrophages of the pulmonary alveoli originate from the cells lining the air sacs. The nature of these cells was analyzed in some detail, and evidence was produced to show that very likely they are not epithelial but mesenchymal in origin. Experimental evidence was cited to the effect that these cells, and not others, are concerned in the defensive and metabolic processes in which the lungs are involved, and that they act in ways similar to the Kupffer cells, the splenocytes and the reticular cells of the bone marrow and the lymph nodes.

It is believed that they represent an essential part of the macrophage system.

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# News and Notes

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**Grants-in-Aid.**—Jean R. Oliver, professor of pathology at the Long Island College of Medicine, has been assigned an additional grant of \$1,000 by the Josiah Macy, Jr., Foundation for continued studies on the pathology of Bright's disease.

The committee on grants-in-aid of the National Research Council at its December meeting, in response to 119 requests, made 36 grants for the support of research projects, of which the following 6 come under the heading of medical sciences: G. Howard Bailey, Johns Hopkins University, "Heterophile Antigens of Bacteria and Plant and Animal Tissues"; Raymond L. Garner, Johns Hopkins University, "Enzymatic Liquefaction of Clotted Human Blood"; R. W. Gerard, University of Chicago, "The Activity of Nerve Tissue and the Central Nervous System"; Balduin Lucké, University of Pennsylvania, "A Neoplastic Disease of the Common Leopard Frog, *Rana Pipiens*"; John R. Paul and James D. Trask, Yale University, "Comparison of Different Strains of Poliomyelitis Virus"; Arthur H. Smith, Yale University, "The Influence of Various 'Inorganic' Ions upon the Body Weight and Blood Changes of Experimental Animals."

**A New Tumor Registry.**—A tumor registry has been established by the medical society in Peoria, Ill. Any physician who registers a case may become a member. For the present all records will be kept in the laboratory of the St. Francis Hospital.

**Prize Paper.**—The eleventh annual prize of \$1,000, given by the American Association for the Advancement of Science to the author of a noteworthy paper presented at the winter meeting, was awarded at Boston to Dr. Reuben L. Kahn, professor of bacteriology at the University of Michigan Medical School, for his paper, "Tissue Reactions in Immunity."

**Society News.**—The American Public Health Association will hold its sixty-third annual meeting in Pasadena, Calif., from September 3 to 6.

The forty-sixth annual meeting of the American Physiological Society, under the presidency of Arno B. Luckhardt, will be held under the auspices of the College of Physicians and Surgeons, Columbia University, from March 28 to 31. The scientific meetings will be held in the Hotel Pennsylvania, which will also serve as headquarters. The demonstrations will be made at the College of Physicians and Surgeons.

Officers of the American Society of Parasitologists have been elected as follows: president, E. E. Tyzzer, Harvard Medical School; vice-president, J. E. Ackert, Kansas State College; secretary, H. W. Stunkard, New York University; treasurer, J. Andrews, Johns Hopkins University School of Hygiene and Public Health.

The Pathological Society of Philadelphia has elected officers for 1934: Morton McCutcheon, president; Esmond R. Long, vice-president; Herbert L. Radcliffe, secretary-treasurer.

The annual meeting of the American Association of Pathologists and Bacteriologists will be held in Toronto, Thursday and Friday, March 29 and 30, 1934. One-half day will be given to a joint meeting with the American Association of Immunologists.

## CLEVELAND SESSION—AMERICAN MEDICAL ASSOCIATION Scientific Exhibit

The Section on Pathology and Physiology of the American Medical Association will sponsor a section exhibit in the Scientific Exhibit at the Cleveland Session, June 11-15, 1934. The section exhibit committee is composed of: W. C. MacCarty, chairman, Rochester, Minn.; Frank W. Hartman, Detroit; A. B. Luckhardt, Chicago, and J. P. Simonds, Chicago.

Applications for space will close on Feb. 26, 1934. Application blanks may be obtained from the members of the committee, or from the Director, Scientific Exhibit, American Medical Association, 535 North Dearborn Street, Chicago.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

SYNOVIAL CELLS IN TISSUE CULTURES. E. VAUBEL, J. Exper. Med. **58**:63 and 85, 1933.

Synovial cultures are differentiated from cultures of other tissues of mesenchymal origin by the type of growth and the function of the cell. In these respects, synovial cultures are more closely allied to chondroblasts and osteoblasts than to fibroblasts. Synovial cells in tissue cultures acquire marked globular cytoplasmic granulations that stain easily with neutral red and sometimes with toluidine blue; they show marked polymorphism with all transitions from round to spindle, polygonal and star shapes, and eventually form an epithelium-like membrane, composed of cells with numerous syncytial bridges. In cultures of typically growing synovial cells a mucin-like substance is elaborated. Typical growth and maximum production of mucin are best maintained in mediums containing a minimum of growth-stimulating substances. Transformation of synovial cell growth into fibroblastic growth is accompanied by a loss of production of mucin. Dying cells apparently do not produce mucin. Amitotic cell division and the formation of macrophage-like cells were observed. A marked tendency to liquefaction of the plasma about the growths was observed and attributed to the elaboration of a proteolytic ferment. The specific designation "synovioblasts" is proposed for these cells.

AUTHOR'S SUMMARY.

TISSUE CULTURES OF FIBROBLASTS. R. C. PARKER, J. Exper. Med. **58**:97, 1933.

Under appropriate conditions, fibroblasts are able to multiply in serum, at a slow rate, for long periods. The rate of multiplication of fibroblasts in a given sample of serum depends entirely on the nature of the strain. Cell races endowed with a high residual growth energy multiply more rapidly in serum than those whose growth potencies are of a lower order. Fibroblasts in the beginning multiply more abundantly in plain serum than in heparinized serum and more abundantly in heparinized serum than in heparinized plasma. Later, these differences become less pronounced. The first effect of serum on fibroblasts is invariably injurious, the degree of injury differing according to the nature of the cell strain and the age of the animal from which the serum is derived. With the passage of time, however, the colonies undergo gradual improvement both in the appearance of the component cells and in their rate of proliferation. In mediums containing the embryonic tissue juice or other growth-activating substances, fibroblasts form colonies that are isomorphic and composed of isomorphic cells. In serum, fibroblasts form colonies of heteromorphic appearance. Each colony becomes composed of cells that differ from one another to a more or less marked degree.

AUTHOR'S SUMMARY.

PREVENTION OF CHOLESTEROL ATHEROSCLEROSIS IN RABBITS. K. B. TURNER and G. B. KHAYAT, J. Exper. Med. **58**:115 and 127, 1933.

In seventeen of nineteen animals, whole thyroid gland, when administered simultaneously with cholesterol, prevented the atheromatous changes produced by the latter in the aorta of rabbits. In this series, thyroxine was less effective, as atherosclerosis occurred in eight of eleven rabbits. Potassium iodide also exerted a strong protective action, as aortic lesions were present in only one of a series of twelve rabbits fed cholesterol and potassium iodide concurrently. The effectiveness of potassium iodide was not shared by potassium bromide or potassium chloride.



A relationship was noted between the level of the cholesterol in the blood and the development of atherosclerosis. In general, the aortic lesions accompanied hypercholesteremia.

Thyroidectomy in itself does not cause a rise in blood cholesterol or the development of atherosclerosis in young rabbits. The feeding of cholesterol produces hypercholesteremia and atherosclerotic lesions in rabbits regardless of the presence or absence of the thyroid glands. Potassium iodide prevents the usual hypercholesteremia and atherosclerosis of the aorta in normal rabbits fed cholesterol, but when the thyroid glands are removed this protective action disappears.

AUTHORS' SUMMARIES.

ARTERIAL DISEASE PRODUCED BY CHOLESTEROL AND VITAMIN D. C. V. HARRISON, *J. Path. & Bact.* **36**:447, 1933.

In experiment 1, cholesterol sclerosis was induced in the rabbit's aorta as a preliminary lesion, and vitamin D sclerosis was later superimposed. In experiment 2, vitamin D sclerosis constituted the preliminary lesion, and cholesterol sclerosis was added. In both cases, the second lesion was confined to the parts of the vessel left unaffected by the first lesion. Both cholesterol sclerosis and vitamin D sclerosis render the affected parts of the vessel wall relatively immobile, and this immobility appears to render the walls less susceptible to the subsequent lesion. It is concluded that the movements of the vessels determine the localization of the lesions due to lack of vitamin D as well as those due to cholesterol, and probably those due to human arterial disease.

AUTHOR'S SUMMARY.

ATHEROSCLEROSIS IN DOGS. W. D. ZINSERLING, *Beitr. z. path. Anat. u. z. allg. Path.* **88**:241, 1932.

The aorta and other organs of twenty-eight dogs, from 8 to 28 years old, were studied. In old dogs, spontaneous lipoidosis of the aorta, the interstitial tissue in the sclera, cartilage, tendons and the fibrous capsule of the internal organs was observed. The lipid was deposited in the interstitial substance, while the fatty degeneration of the tissue cells played a minor rôle. These changes were identical with those observed in man and the rabbit. Characteristic for the dog is the secondary lipoidosis in fibrotic areas of the intima, in contradistinction to the primary lipoidosis of the human aorta. In man and in the rabbit, fibrosis of the aortic wall follows atheromatous degeneration. In the dog, cholesteremia does not play the same important rôle of a causative factor of lipoidosis of the aorta as it does in the rabbit. It was impossible to produce lipemia and atherosclerosis in dogs by feeding an excess of cholesterol. In old dogs, endogenous factors seem to produce a disturbance of the cholesterol metabolism which, together with the fibrosis of the aortic wall, must be regarded as the essential cause of atherosclerosis.

C. ALEXANDER HELLWIG.

HEMATOPOIESIS IN THE AXOLOTL AFTER REMOVAL OF THE EXTERNAL ZONE OF THE LIVER. B. T. MALYSCHEW, *Beitr. z. path. Anat. u. z. allg. Path.* **88**:315, 1932.

In the axolotl, formation of the granulocytes occurs normally in the external zone of the liver, while the spleen produces the red blood cells. After removal of the external zone of the liver, the reticulo-endothelial cells lining the muscle trabeculae of the heart and the Kupffer cells of the liver produce hemocytoblasts. The latter develop into granulocytes and erythroblasts. Some of the newly formed hemocytoblasts are carried to the epicardium and form there a marginal zone of hematopoiesis, with neutrophil and eosinophil granulocytes predominating. The external zone of the liver regenerates from Kupffer's cells and their descendants, the hemocytoblasts, whereas the parenchyma proper of the liver is regenerated by mitotic division of liver cells.

C. ALEXANDER HELLWIG.

THE INFLUENCE OF FOOD ON THE HEALING PROCESS OF WOUNDS IN THE STOMACH. E. L. HOWES, Beitr. z. path. Anat. u. z. allg. Path. **88**:435, 1932.

The healing of surgical wounds in the stomach was studied in three groups of white rats. After operation the first series received a well balanced diet; the second series, one half of the volume and of the caloric value given the first, and the third series, three fourths of the caloric value of the stock diet, the volume being brought up to normal by the addition of sawdust. A diet containing only half of the regular volume and half of the caloric value did not interfere with the process of healing. In the third series, however, the process of healing was definitely retarded. Since this group of white rats received a diet which was of higher caloric value and more voluminous than that of the second group, the irritating sawdust in the diet must have been the responsible factor. Postoperative diet, therefore, should be completely free from irritating substances.

C. ALEXANDER HELLWIG.

CONSTITUTIONAL THROMBOPATHY (A NEW DISEASE OF THE BLOOD). E. A. WILLEBRAND and R. JÜRGENS, Klin. Wchnschr. **12**:414, 1933.

The authors studied thirty-five "bleeders" from a group of three families with bleeders for four generations. The results are believed to justify the establishment of a new group in the classification of diseases of the blood. Constitutional thrombopathy is a dominant, sex-linked, hereditary disease, occurring more frequently in females than in males and characterized by spontaneous bleeding from the skin, mucous membranes and wounds. The blood platelet count is normal. However, the bleeding time is markedly prolonged. The plasma proteins are normal. The authors ascribe the disease to hypofunction of the blood platelets, and particularly to a diminution in the agglutination of thrombocytes and deficiency in thrombus formation. The condition is distinct from hemophilia and thrombopenia.

J. KLEIN.

DIAPHRAGMATIC ANGINA PECTORIS. L. HOFBAUER, München. med. Wchnschr. **80**:411, 1933.

The author is of the opinion that attacks of angina pectoris may be due to diseases of the diaphragm, such as hernia and empyema, and cites clinical records to support his contention. Hyperesthesia of the diaphragm may cause pain, dyspnea and a feeling of constriction in the chest. When there is no involvement of the diaphragm, the diaphragm may nevertheless become hyperesthetic as the result of changes in the circulatory organs (the coronary arteries, aorta and myocardium) caused by a visceromotor reflex.

J. KLEIN.

ORIGIN OF BONE MARROW GIANT CELLS IN EXTRAMEDULLARY MYELOPOIESIS. R. P. CUSTER, Virchows Arch. f. path. Anat. **288**:213, 1933.

In a series of experiments undertaken to study the effect of reticulo-endothelial blockade on extramedullary myelopoiesis, marked formation of bone marrow giant cells in the spleen was noted in one animal. In lesser degree the phenomenon was also evident in the liver and the lymph nodes. In this animal, india ink had been used to form the blockade and sapotoxin to cause anemia. The bone marrow appeared inactive and degenerated. In another rabbit that had also received sapotoxin, but in which carmine had been used for blockade, the bone marrow was very active and contained many hyperplastic giant cells. The spleen, liver and lymph nodes contained few giant cells. The author interprets his findings as indicative of the local extramedullary formation of the giant cells and postulates the action of a necrohormone as a factor in the process.

O. T. SCHULTZ.

LOCAL CIRCULATORY REACTIONS OF THE SKIN. E. REINHARDT and G. RICKER, *Virchows Arch. f. path. Anat.* **288**:393, 1933.

The effects of local mechanical and chemical stimuli on the vessels of the skin, as determined by capillary microscopy, form the basis of an extended theoretical discussion of the resulting changes and their interpretation and of a critical survey of the existing theories relied on to explain the phenomena noted. The various stimuli, regardless of their nature, evoke a diphasic reaction, which consists in ischemic anemia followed by peristaltic hyperemia of the papillary vessels. The hyperemia of stronger stimulation may lead to exudation and the formation of wheals. Maximal stimulation leads to stasis without exudation. The various reactions are the expression of the degree of action and not of the quality of the stimulus. They result from the action of the stimulus on the constricting and dilating nerves of the vessel. The action may be either direct or indirect, i. e., reflex. The place of action may be any part of the nervous system from the local vessel to the cerebral cortex, and the direction of the stimulus may be either centripetal or centrifugal. The stimulus which manifests itself in a local vascular reaction of the skin may therefore be psychic, mechanical, physical or chemical. Whatever the character of the stimulus, the vascular response is of the same character, varying only with the degree of action of the stimulus. The older cellular theory of vascular physiology, which postulates an action directly on the cells of the vessels, is held to be untenable. The more modern doctrine of humoral vascular physiology according to which soluble substances in the blood or tissue fluids mediate the vascular reactions is also held to be untenable. O. T. SCHULTZ.

FUNCTION OF THE RETICULO-ENDOTHELIAL SYSTEM IN EXPERIMENTAL STREPTOCOCCIC SEPSIS. J. VOICU, A. VITÁLYOS and L. BOER, *Virchows Arch. f. path. Anat.* **288**:455, 1933.

Rabbits were infected with streptococci isolated from puerperal fever. In overwhelming, rapidly fatal experimental infections, there was no formation of opsonins, agglutinins or bacteriotropins. The reticulo-endothelial system of the animals revealed no reactive changes, and its cells had only weak properties of vital storage. In less severe infections, opsonins, agglutinins and bacteriotropins appeared in the blood. In animals killed during this state, the reticulo-endothelial system revealed hypertrophy and hyperplasia of its cells, which exhibited greater activity for vital storage than is normal. The formation of immune bodies and the hyperplasia and hyperactivity of the reticulo-endothelial system cease when the resistance of the animal is overcome by the infecting organism. The authors conclude that the study of the reactions of the reticulo-endothelial system is an excellent method for evaluating the therapeutic efficiency of remedial agents.

O. T. SCHULTZ.

TOXIC EFFECTS OF IRRADIATED STEROLS. B. KELLNER, *Virchows Arch. f. path. Anat.* **288**:491, 1933.

The histologic effects of irradiated sterols were studied in two series of fully grown young rats comprising, respectively, 85 and 130 animals. It is of interest that crystalline viosterol was found to be nontoxic in the first series of animals, although the substance is therapeutically active in rickets in man. This led to a study of the daily dosage of several irradiated sterols of ergot origin necessary to cause toxic effects. Subtoxic doses caused no histologic changes, even when continued for a long time, to half a year. The toxic effects noted were those previously described, namely, degeneration and necrosis followed by calcification. The kidney and aorta were most frequently affected. The peripheral arteries, heart muscle and voluntary muscle came next. In the bones toxic doses led to the formation of osteoid tissue, followed in some animals by hypertrophic changes in the bones and in others by atrophic changes, the latter being noted especially with the larger doses or a more prolonged administration. O. T. SCHULTZ.

RELATION BETWEEN THE ENDOCRINE GLANDS AND THE ESTRUS CYCLE. S. TSUCHIMOTO, *Jap. J. Exper. Med.* **11**:129, 1933.

The subcutaneous transplantation of the anterior lobe of the hypophysis of the guinea-pig or the rabbit caused ovulation in immature white rats. The genital organs showed early maturity; ovarian follicles developed and matured, changed into corpora lutea or formed cysts. The anterior lobe had no influence on ovulation in maturity. The central and posterior lobes caused no change in the estrus cycle, and the anterior lobe failed to prevent shrinkage of the genital organs after ovariectomy.

J. KLEIN.

### Pathologic Anatomy

DEGENERATION OF THE CORPUS CALLOSUM IN HYDROCEPHALUS. N. A. ZOLOTOWA, *Virchows Arch. f. path. Anat.* **280**:343, 1931.

In two cases of internal hydrocephalus (one syphilitic, the other congenital) in infants the corpus callosum was found to be markedly atrophied. Primary failure of development and atrophy secondary to the hydrocephalus are possible causes. However, the anlage of this structure develops after the fourth month of pregnancy. The occurrence of internal hydrocephalus before this time interferes with its development, resulting in a rudimentary type.

PERRY J. MELNICK.

ACROCEPHALOSYNDACTYLISM (APERT). ARNOLD FLINKER, *Virchows Arch. f. path. Anat.* **280**:546, 1931.

The author reviews the nine cases of acrocephalosyndactylism gathered from the literature by Apert, and adds a tenth. The condition is characterized anatomically by syndactylism of all four extremities and a characteristic deformity of the head consisting of acrocephaly, a peculiar outline of the forehead and flattening of the occipital region. There are other variable congenital anomalies. The condition is not hereditary or familial. The author's patient came from a cretin population and had a large goiter and myxedema.

PERRY J. MELNICK.

LIPOID NEPHROSIS. A. R. KANTROWITZ and PAUL KLEMPERER, *Virchows Arch. f. path. Anat.* **280**:554, 1931.

Using histologic methods recommended by McGregor which bring out finer morphologic changes than does the hematoxylin-eosin stain, Bell came to the conclusion that lipid nephrosis is primarily glomerulitis. The authors studied two cases of undoubted clinical and pathologic lipid nephrosis by these methods. Proliferation of glomerular endothelium or of capsular epithelium, the criterion of glomerular inflammation, could not be found. The cells of these structures were swollen and contained lipid droplets, changes which were not so definite with the hematoxylin and eosin stain. However, these changes are considered not to be inflammatory but to be phenomena of degeneration and lipid storage, part of the picture of lipid nephrosis.

PERRY J. MELNICK.

CHANGES IN THE ELASTIC TISSUE OF THE LUNG IN A CASE OF BRONCHIAL ASTHMA. W. WAWERLA, *Virchows Arch. f. path. Anat.* **285**:12, 1932.

In a man, aged 36, who had had bronchial asthma for twenty-four years, histologic examination of the lungs revealed some unusual changes in the elastic tissue. The elastic fibers of the terminal arterioles and of the smaller bronchi were ruptured, swollen and encrusted with iron and calcium. About many of the arterioles there had been formed foreign body granulomas that compressed and occluded the vessels. In the pulmonary parenchyma the elastic tissue framework was in places hypertrophied and in other places broken and torn, and in still other areas elastic tissue had disappeared. Occlusion of the small arteries had led to

hypertrophy of the right side of the heart. Engorgement of the vessels and pathologic functioning of the lung are held responsible for the changes in the elastic tissue.

O. T. SCHULTZ.

UNUSUAL FATAL INTESTINAL HEMORRHAGE. H. WEBER, *Virchows Arch. f. path. Anat.* **285**:46, 1932.

A woman, aged 50, was found, a year before her death, to have tertiary syphilids of the skin and cerebrospinal syphilis that yielded to antisyphilitic treatment. Syphilitic cirrhosis of the liver developed, and she died from a sudden massive intestinal hemorrhage. At necropsy there was found a ruptured submucous varix of the lower portion of the ileum. This portion of the intestine was adherent in a hernia of the scar of a previous laparotomy. The unusual situation of the ruptured varix was due to the interference of the adhesion with the collateral circulation, which everywhere else was well developed.

O. T. SCHULTZ.

QUANTITATIVE STUDY OF OVARIAN FOLLICULAR ATRESIA. W. BLOTEVOGEL, *Virchows Arch. f. path. Anat.* **285**:53, 1932.

By means of serial sections of both ovaries of sixteen white mice and five primates (*Cynocephalus hamadryas*), the total number of primary, secondary and atretic follicles was determined and the volume of follicular tissue in relation to the volume of the entire ovary was estimated. In both the species studied there was great variation in the number of follicles in animals of the same species, in the number of follicles in the two ovaries of the same animal and in the size of the ovaries. In each species about half of the follicles were undergoing atresia. In the primate there was a correlation between the number of follicles and the volume of the ovary; in the mouse no such correlation could be detected. In neither species was there a correlation between the number or the volume of atretic follicles and the volume of the ovary, indicating that follicular atresia is not the result of overcrowding and lack of space within the ovary. This conclusion forms the basis of a brief discussion of inherent lethal factors within the germ cell, as opposed to environmental factors. The inherent lethal factors manifest their effects even before fertilization. In the species studied, approximately half of the germ cells are destined to destruction within the ovary.

O. T. SCHULTZ.

CONGENITAL MALDEVELOPMENTS OF THE NOSE. B. LANG, *Virchows Arch. f. path. Anat.* **285**:93, 1932.

Two unusual examples of nasal maldevelopment are described, one of proboscis lateralis and one of Kundrat's cebocephalic form of arhinencephaly. In each there were multiple malformations in addition to the nasal one, to which special attention is given. In the case of proboscis lateralis, the left half of the nose was normally formed. The right half was represented by a short, sessile structure attached at the inner canthus of the right eye. The right nasal bone and the right nasal cavity were absent. There was a coloboma of the right lower eyelid. The intermaxillary bone was absent. In the case of cebocephaly a rudimentary nose with a single nostril had been formed. The following structures had not been formed: the horizontal and vertical plates of the ethmoid, the small wings of the sphenoid, the optic foramina, the vomer, the lacrimal bones, the intermaxillary bone and the nasal cavities. The frontal lobes of the brain were fused in the midline, with maldevelopment of the olfactory lobes and other parts of the brain. The endogenous and exogenous factors that may have a part in the genesis of such maldevelopments, and the period of embryologic development at which the latter may arise, are discussed.

O. T. SCHULTZ.

CONGENITAL VERTEBRAL DEFORMITY WITH ABSENCE OF THE NECK. A. FELLER and H. STERNBERG. *Virchows Arch. f. path. Anat.* 285:112, 1932.

In the fourth of a series of studies of congenital malformations of the vertebral column, the authors describe six cases of absence or shortness of the neck. Five of these occurred in fetuses and one in a woman who died at the age of 51 years. The anomaly was described by Kippel and Weil in 1912 and is referred to in the German literature as the Kippel-Weil syndrome. In the authors' cases there were various malformations of the cervical and upper thoracic vertebrae, including failure of development or rudimentary development with fusion of vertebrae. Common to all was a defect of the dorsal arches of the vertebrae, which continued upward into the occipital bone. The anomaly is ascribed to interference with the normal development of the primitive segments of the body; it manifests itself before cartilage has been formed in the anlagen of the vertebrae.

O. T. SCHULTZ.

HISTOLOGY OF UNDULANT FEVER. F. WOHLWILL, *Virchows Arch. f. path. Anat.* 286:141, 1932.

A woman, aged 67, died suddenly at the end of the fourth week of an attack of undulant fever. Death was due to pulmonary embolism; the embolus came from a thrombus of the femoral vein. The spleen was enlarged. Microscopic examination revealed numerous minute cellular nodules composed of epithelioid reticulo-endothelial cells. Intermingled with these were a few giant cells and a few plasma cells. The small granulomas were situated in the malpighian bodies, only a few being found in the pulp. The para-aortic and bronchial lymph nodes and the bone marrow contained similar minute granulomas. In the liver were numerous focal necroses, about some of which there was a granulomatous reaction similar to that of the spleen and lymph nodes. In the small number of cases of undulant fever in which necropsy has been performed, death has been due to some other condition and no changes characteristic of infection with *Bacillus abortus* have been noted. In Wohlwill's case also death was due to a condition other than the infection, but it occurred at a time when lesions that the author considers characteristic of the disease were still present.

O. T. SCHULTZ.

INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM IN A CASE OF GENERALIZED XANTHOMATOSIS (SCHÜLLER-CHRISTIAN'S DISEASE). H. CHIARI, *Virchows Arch. f. path. Anat.* 288:527, 1933.

Previous reports of Schüller-Christian's disease have presented clinical or anatomic evidence of only slight involvement of the central nervous system. Chiari reports a careful histologic study of the brain in a case of the disease with marked involvement of the central nervous system. A man who died at the age of 27 had had osteomyelitis of the right tibia at the age of 20. Operation was followed by recovery. Five years before death the symptoms of diabetes insipidus appeared and persisted until death. Four years before death areas of softening were noted in the occipital bone; roentgenologic examination of these areas a year before death led to a clinical diagnosis of Schüller-Christian's disease, which was confirmed by biopsy. Headache, weakness of the extremities, difficulty in standing and walking, abnormalities of sight and hearing and projectile vomiting indicated severe and progressive involvement of the central nervous system. Death was due to cavernous pulmonary tuberculosis. Necropsy and the subsequent microscopic examination brought to light no areas of the characteristic cholesterol-infiltrated tissue in the thoracic or abdominal organs. Such tissue was, however, present in the ilium, femur, cranium, dura, brain and cervical cord. In the brain the xanthomatous tissue formed macroscopically visible yellowish areas in the cerebrum and cerebellum. These areas were situated in the white matter, chiefly about the ventricular system, and spared the gray matter of the cerebrum. The cortex of the cerebellum was involved. The yellow tissue was composed of vacuolated

foamy cells the cytoplasm of which contained doubly refractile droplets that stained with sudan III. The cells were of two types. Larger cells were of mesenchymal origin and were derived from the tissue about the blood vessels. Smaller lipoid-containing cells were derived from the microglia and oligodendroglia. The macroglia did not participate in the process. In the areas of xanthomatous tissue the myelin sheaths and axis cylinders had disappeared. Except in the cortex of the cerebellum and the anterior horns of the cervical cord, the ganglion cells contained no lipid.

O. T. SCHULTZ.

HISTOPATHOLOGY OF THE MARGINAL NODULES CAUSED BY PROSTHESIS OF THE LOWER LIMB. F. WOHLWILL, *Virchows Arch. f. path. Anat.* **288**:576, 1933.

Zur Verth first described nodules that develop in the skin of the inner surface of the thigh where the margin of an artificial lower extremity makes pressure. Arising subepidermally, they lead to ulceration of the covering epidermis and to the formation of small abscesses, which may persist as chronic fistulas. According to the histologic studies of zur Verth and others, the lesions are foreign body granulomas that develop about fibers of leather that are forced into the skin by pressure. Wohlwill examined histologically twenty lesions of the kind under consideration. In three the granulomas contained bodies the foreign character of which could not be definitely determined; origin from the stroma of clumped red corpuscles is considered possible. In the remaining cases foreign bodies surrounded by giant cells were present, but in only one of these was the foreign body held to be actually foreign to the tissue. In the other instances the genesis of the foreign bodies could be traced to the hornified epithelium of hair follicles, which had undergone hypertrophy and hyperkeratosis as the result of pressure and moisture.

O. T. SCHULTZ.

CHRONIC ABSCESS OF THE THYROID. K. STOJALOWSKI, *Virchows Arch. f. path. Anat.* **288**:660, 1933.

The avascular character of the capsule protects the thyroid against infection by direct continuity from adjacent tissues. Infection of the gland usually occurs by way of the blood or lymph stream. In one of the two cases here reported the abscess was single and was surrounded by a thick fibrous wall. The infection originated in the tonsil. In the second case the abscesses were multiple, were surrounded by fibrous tissue and were secondary to an ulcerated epithelioma of the larynx.

O. T. SCHULTZ.

RELATION OF MICROLITHS IN THE BILE TO CHANGES IN THE LIVER AND GALLBLADDER. W. BÜTTNER and G. LEMMEL, *Virchows Arch. f. path. Anat.* **288**:682, 1933.

Bile removed from the gallbladder at necropsy was examined microscopically for microliths. The attempt was made to correlate the occurrence of the latter with histologic changes in the liver and gallbladder. In 800 consecutive necropsies microliths were detected 75 times (9.4 per cent). The incidence of their occurrence increases with age and stasis or stagnation of bile, but simple mechanical stagnation is not a factor in their formation. Inflammation of the gallbladder with resulting stagnation of bile may lead to the formation of microliths. There was no parallelism between cholesterol infiltration of the mucosa of the gallbladder and the formation of microliths. The formation of microliths was associated most often with pathologic alterations of the liver cells of variable degree, but there is no uniform pathologic background for this formation. Microliths were seen more often in light-colored bile than in deeply colored specimens, and more often when the quantity of bile in the gallbladder was small than when it was large, indicating that disturbance in biliary secretion is a factor in the formation of microliths.

O. T. SCHULTZ.

RELATION OF RHEUMATIC INFECTION TO ARTERIOSCLEROSIS OF THE AORTA.  
M. SCHULZ and F. KLINGE, *Virchows Arch. f. path. Anat.* **288**:717, 1933.

The thirteenth contribution of Klinge and his co-workers to the pathologic changes in rheumatic infection is a profusely illustrated article of sixty-three pages that attempts to evaluate the rôle of rheumatic infection in arteriosclerosis of the aorta. The vascular pathologic process of the active stage of the disease was described in the fourth communication of the series. The present article begins with a brief description of these early changes in five additional cases in which the patients died while active rheumatic lesions were still present. It then proceeds with its main thesis, a study of the relation of aortic arteriosclerosis to rheumatism and a variety of other diseases. For this study 188 aortas were selected that revealed macroscopic evidence of arteriosclerosis. Each aorta was examined microscopically throughout its length by being rolled into a spiral and embedded in a single pyroxylin block. For special histologic studies smaller blocks were excised and examined in frozen and paraffin sections. According to the gross pathologic and clinical manifestations of the disease process, the material is divided into the following ten groups: (1) 12 cases of endocarditis or lesions of the joints of certain rheumatic origin; (2) 15 cases of endocarditis of probable rheumatic origin; (3) 24 cases of nonrheumatic endocarditis; (4) 20 cases of syphilis; (5) 10 cases of chronic infection of various kinds; (6) 26 cases of clinical hyperpiesis; (7) 19 cases of acute infection; (8) 7 cases of septicopyemia; (9) 13 cases of tuberculosis; (10) 43 cases of diffuse atheromatosis and arteriosclerosis of the aorta. For each group a tabulation gives briefly the gross characteristics of the aorta, the localization of the process and the changes in the media and adventitia for each case. Then follows a more detailed description of selected cases, which is succeeded by a succinct summary of the findings for the group. At the end of the article is a brief discussion of the relation of the findings to some moot problems in the genesis of arteriosclerosis. The focal character of the lesions of the media in many of the groups is accepted as evidence of primary damage to the media, with secondary hyperplasia and later degeneration of the intima. But the occurrence of swelling and fibrinoid degeneration of the ground substance of the intima, with no underlying medial alteration, is sufficient proof that the arteriosclerotic process may be initiated also by primary disease of the intima. Severe damage to the media occurs not only in syphilis, but in rheumatism and in a variety of other chronic diseases. Aortic disease of rheumatic origin may involve any part of the aorta, but was noted most frequently in the abdominal aorta. In rheumatism and in nonrheumatic chronic endocarditis, foci of lymphocytic infiltration and vascularized scars were frequently seen in the media. Acute infection of any kind may activate such older lesions of the aorta. Mucoid degeneration of the media was noted in a wide variety of chronic diseases, but was most marked in rheumatism. Deposition of calcium occurred in a wide variety of diseases, but was more marked in tuberculosis than in any other group. Streaks of avascular fibrosis in the media without any inflammatory reaction were seen most often in the aortas of the patients who had had high blood pressure. In tuberculosis there was not seen the inflammatory involvement of the media that was so frequently observed in rheumatism and other chronic diseases. The diagnosis of rheumatic aortic sclerosis may be made at the postmortem table from the gross appearance of the aorta, the localization of the process and a history of rheumatic infection. Even if one is unwilling to accept all the deductions and conclusions that Klinge has drawn from the work presented in this series of contributions, one must admire the volume of work on which the conclusions are based, the thoroughness with which the work has been done, the planned orderliness of the work and the concise, organized manner in which the work is presented in printed form.

O. T. SCHULTZ.



UNUSUAL HISTOLOGIC FORMS OF RHEUMATIC INFECTION. R. RÖSSLE, *Virchows Arch. f. path. Anat.* **288**:780, 1933.

Rössle begins with a discussion of the question whether rheumatic infection is a disease of one or more organs, which is the older view, or a systemic disease of the mesenchyme involving widely the connective tissue and blood vessels, which is the concept of Klinge and other contemporary observers. The widespread involvement of the mesenchymal system of tissues can be explained only on the basis of an allergic state of these tissues. That febrile rheumatism is a disease characterized by hyperergic inflammation of the mesenchyme Rössle considers established. Variations in the degree and localization of the hyperergic reaction depend, in part, on differences in the degree of sensitization of the mesenchyme, on differences in the physiologic function of the connective tissue and blood vessels of the organs and tissues and on differences in the mechanical activity of the tissues. As a contribution to the conception that rheumatic infection is a mesenchymal systemic disease, he describes and discusses in detail five cases that differed widely from each other in their clinical and pathologic histologic manifestations, but all of which he would include in the broad group of rheumatic disease. The first case was one of classic rheumatic polyarthritis, with widespread vascular involvement. The second was a case of less active polyarthritic disease, in which there was marked reaction of the blood vessels of the synovial and joint tissues; the descriptive term applied to this case is chronic polyarthritis vasculosa. The third case was one of obliterating endophlebitis of the liver. The fourth case was one that began with pains in the joints and ended in sepsis; there was marked arteritis, with some lesions that were rheumatic and others that resembled periarteritis nodosa. The fifth case, one of rheumatic periarteritis, Rössle calls tuberculoid periarteritis nodosa. The periarterial granulomatous lesions of the spleen, liver and kidney had led to a gross anatomic diagnosis of miliary tuberculosis. The lesions had the histologic structure of giant Aschoff bodies. Similar smaller lesions were present in the myocardium. From the histologic study of this series of cases Rössle concludes that the limits of what is to be included under rheumatic infection must be broadened to include a wide variety of mesenchymal reactions.

O. T. SCHULTZ.

HEALING STAGES OF PERIARTERITIS NODOSA AND THEIR RELATION TO JUVENILE ATHEROSCLEROSIS. E. JÄGER, *Virchows Arch. f. path. Anat.* **288**: 833, 1933.

Healed lesions of periarteritis nodosa are rarely seen, because the disease is usually a continuous and progressive process. In three men who died at the ages of 37, 30 and 39, the clinical duration of the illness was thirty-seven, fourteen and five months, respectively. In the first case a clinical diagnosis of periarteritis nodosa had been made early in the course of the disease. In each case necropsy revealed marked atherosclerosis. Microscopic study brought to light the changes in the small arteries which Jäger describes as the healed and healing lesions of periarteritis. In the case of shortest duration active lesions were also present. Injury to the media leads to hyperplasia of the overlying intima in the form of localized nodular thickenings. A single vessel may reveal several intimal nodules of different ages. Injury to the wall during the active stage may lead to the formation of miliary aneurysms; such aneurysms were seen especially in the coronary arteries. In the organization of thrombosed arteries or aneurysms, blood channels are formed; about these there develop a new elastic membrane and a new media. The intimal hyperplasia bears no direct relation to lipoid infiltration, although the intimal nodules may sometimes be formed over what were originally superficial areas of lipoid infiltration. The formation of the intimal callus is not the result of atherosclerosis; when atheromatous degeneration occurs in the callus it is the result of changes in the ground substance. Nodose atherosclerosis in younger persons, when associated with epicardial proliferation over the nodular areas of the coronary arteries and with scars of infarcts in the spleen and kidney, should suggest the possibility of healed periarteritis nodosa.

O. T. SCHULTZ.

CHANGES IN THE NERVES, SKIN AND BONES IN RECKLINGHAUSEN'S NEUROFIBROMATOSIS. A. STALMANN, *Virchows Arch. f. path. Anat.* 289:96, 1933.

This investigation is based on the clinical study of thirty-five cases of neurofibromatosis, brief summaries of which are presented. Whenever possible, lesions of the skin, nerves and bones were removed during life and subjected to histologic study. One case came to necropsy. Pigmentary anomalies of the skin are the most common lesions, and are usually of such histologic character that they can be distinguished from other pigmentary nevi. The cutaneous tumors are mesenchymal overgrowths that may arise in connection with the nerves of the skin. Keloids develop in scars as the result of a congenital predisposition of the mesenchyme to overgrowth. The lesions of the peripheral nerves described are the neurinoma and the plexiform neuroma. The lesions of the bones are dystrophic, and are the result of softening followed by deformity. Destruction of bone with pseudarthrosis is frequent in childhood. Psychic disturbances are the result of the participation of the central nervous system in the disease process. Changes in the organs of internal secretion are ascribed to the dystrophic action of the vegetative nervous system. Neurofibromatosis is held by the author to be a blastomatous disease of the nervous system, which has its origin in the embryonic period of life. Abnormal impulses from the diseased nervous system cause functional and anatomic abnormalities of the various organs, the various lesions of which are part of the disease itself.

O. T. SCHULTZ.

HYPERTROPHY OF THE ESOPHAGUS AND STOMACH IN A CASE OF RECKLINGHAUSEN'S NEUROFIBROMATOSIS. H. J. SCHERER, *Virchows Arch. f. path. Anat.* 289:127, 1933.

The localized hypertrophy of organs or limbs that is not infrequently associated with neurofibromatosis is held by some to be a concomitant but independent maldevelopment, and by others to be an essential part of the disease due to trophic disturbances through involved nerves. The condition here reported occurred in a man who died at the age of 53 as the result of hemorrhage from an acute ulcer of the cardiac end of the stomach. The external manifestations were those of typical neurofibromatosis. Internal examination at necropsy revealed fusiform and nodular enlargement of both vagus nerves and marked thickening of the wall of the esophagus and stomach. The appendix was abnormally long and thick. Microscopically, the thickening of this organ was due to neurinomatous hyperplasia of the submucosa. Increase in the thickness of the wall of the esophagus and stomach was due to hyperplasia of the circular and longitudinal muscle, without an abnormal increase in the nerve elements. The axis-cylinders of the vagi were separated by neurinomatous proliferation, but were not interrupted or appreciably damaged, although they exhibited slight fusiform enlargements. The author interprets the muscular hypertrophy of the esophagus and stomach as the result of abnormal stimuli that reached these organs through the altered vagus nerves.

O. T. SCHULTZ.

RECURRENT ENDOMETRIOSIS OF LAPAROTOMY SCAR. M. VERSÉ, *Virchows Arch. f. path. Anat.* 289:186, 1933.

This is a brief report of an endometrial nodule that developed in a laparotomy scar three fourths of a year after a similar nodule had been excised from the scar. The first nodule was removed one and three-fourths years after laparotomy and an operation on the uterus.

O. T. SCHULTZ.

EPIDERMAL CYSTS FOLLOWING THE INJECTION OF IRRADIATED ERGOSTEROL. H. SANDER, *Virchows Arch. f. path. Anat.* 289:190, 1933.

Epidermal cysts developed at the site of injection of irradiated ergosterol into the vein of the ear in thirteen of twenty-two rabbits. The cysts were derived from hair follicles and sebaceous glands. Ergosterol that had not been irradiated did not have a similar effect.

O. T. SCHULTZ.

COMPARISON OF COMPENSATORY HYPERTROPHY OF THE THYROID GLAND WITH THAT FOLLOWING THE ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT. MARTHA SILBERBERG, *Virchows Arch. f. path. Anat.* **289:201**, 1933.

Previous work had shown that the intraperitoneal injection of an extract of the adenohypophysis leads to hypertrophy of the thyroid gland characterized by a change in the character of the colloid, multiplication of the follicular epithelium and a change in the character of the epithelium. The basal metabolic rate is increased. The compensatory hypertrophy that follows the removal of a lobe is characterized by an increase in the number of epithelial cells, with no change in their character. The growth stimulus of resection of a lobe is therefore held to be purely quantitative in its effect, whereas the stimulus of pituitary extract is qualitative in its effect. The purpose of the present experiments, which were carried out on guinea-pigs, was to determine the influence of the two kinds of growth stimuli when applied simultaneously or one after the other. The experiments consisted of a control series to determine the characteristics of compensatory hypertrophy alone; a series in which resection was followed by the administration of pituitary extract, with variable periods of rest before the thyroid was subjected to examination; a series in which resection followed the administration of the extract, and a series in which resection followed the injection of the extract and was succeeded by another series of injections of extract. In the normal animal the qualitative effects of the injection of pituitary extract reach their maximum after six injections, and the quantitative effects of compensatory hypertrophy reach their maximum at the end of eighteen days. When the extract was administered immediately following resection of a lobe of the thyroid gland, there was no summation of the two effects, but the qualitative effect of the pituitary extract was held in abeyance until the quantitative effect of resection had run its normal course. If the administration of extract was continued for only a short period after resection, the completion of the compensatory hypertrophy was delayed. The maximum degree of change resulted when the compensatory hypertrophy of a thyroid previously stimulated by pituitary extract was allowed to run its course and another series of injections was then given. The quantitative and qualitative growth stimuli interfere with each other, but by the proper alternation of periods of rest and of stimulation the maxima of the quantitative and qualitative effects can be made to coincide, resulting in a thyroid that has the histologic structure of the abnormally functioning hyperplastic thyroid of exophthalmic goiter.

O. T. SCHULTZ.

DWARFISM DUE TO CONGENITAL SYPHILIS OF THE HYPOPHYSIS. RUTH KATZENSTEIN, *Virchows Arch. f. Path. Anat.* **289:222**, 1933.

A woman, 131 cm. in height, with the stigmas of congenital syphilis, died at the age of 51 of cerebral hemorrhage. Growth had been retarded since the third year of life; she was backward in mental development; she had never menstruated, and since the age of 30 she had had disturbance of locomotion and vision of a progressive character. At necropsy the external and internal genitalia were found to be rudimentary, the ovaries apparently never having functioned. The sella turcica and the hypophysis were small. The hypophysis had a thick fibrous capsule; the posterior lobe appeared normal, but the anterior lobe contained only remnants of glandular tissue in its peripheral portion and these were separated by broad bands of connective tissue. The remnants of the adenohypophysis contained no basophil cells. There were no active inflammatory changes in the pituitary gland; the changes noted were ascribed to congenital syphilis which had run its course. The thyroid gland was small and fibrous. The internal organs were hypoplastic. The author considers the hypophyseal involvement the basic condition. It led to retardation of growth in length, and to atrophy of the thyroid and ovaries. The changes in the latter organs led to failure of development of the genitalia and to hypoplasia of the other internal organs.

O. T. SCHULTZ.

PLACENTAL CHANGES IN SYPHILIS. T. E. OLIN, Arb. a. d. path. Inst. d. Univ. Helsingfors 6:377, 1931.

These studies are based on thirty-nine cases of syphilitic infection. Microscopic study demonstrated leukocytic infiltration of the decidua basalis at the marginal portion of the placenta. Diffuse infiltration was noted elsewhere in the decidua and in the intervillous septums. Hyperplasia of the villi was observed in the chorion frondosum, as were leukocytic infiltration and abscesses of the villi. The latter occur in two forms: (1) abscesses showing proliferative changes in the stroma with partial destruction of the syncytium (the production of fibrin, overgrowth of collagenous connective tissue and even giant cells may be noted); (2) the exudative type, in which the infiltrated villi fuse and combine with other tissues (macroscopically, the center of the inflamed area may become soft and form abscess-like cavities; edema of the villi is often present). The blood vessels show endothelial proliferation, thickening of the intima and, frequently, obliteration with lymphocytic infiltration. Leukocytic infiltration of the chorion is comparatively frequent. It affects the chorionic cells, the walls of the blood vessels and the stroma. There is usually associated inflammation of the amniotic membrane.

The chief changes in the umbilical cord are in the blood vessels, which show endothelial proliferation, lymphocytic infiltration, even into Wharton's jelly, and destruction of the elastic coat. The veins are more frequently and severely involved than the arteries.

Spirochetes were demonstrated in the placenta in three of the thirty-nine cases.

Antisyphilitic treatment may inhibit to a high degree most of the pathologic reactions. In general, it is concluded that one may be fairly sure that there is syphilitic infection of the placenta when hyperplasia of the villi, miliary abscesses, inflammatory changes in the cord and vascular lesions are present.

J. KLEIN.

### Microbiology and Parsitology

GROWTH OF CLOSTRIDIUM BOTULINUM ON SYNTHETIC MEDIUM. W. BURROWS, J. Infect. Dis. 52:126, 1933.

Evidence supporting the hypothesis of essential amino-acids for bacteria is presented. Certain strains of *Clostridium botulinum* grew well on certain synthetic mediums. The amino-acids cystine, leucine and proline were essential to the growth of the strains of *Cl. botulinum* used. The amino-acids lysine and glycine, while not essential, were active in promoting growth of these strains of *Cl. botulinum*. Isoleucine and oxypoline could be substituted for leucine and proline, respectively, as essential amino-acids. The obligate anaerobes, *Clostridium welchii*, *Clostridium chauvæi*, *Clostridium sporogenes*, *Clostridium histolyticum* and *Clostridium tetani* would not grow on a simple sympathetic medium which would support the growth of these strains of *Cl. botulinum*. The type A culture of *Cl. botulinum* used showed better growth on these mediums than either of the two type B cultures.

AUTHOR'S SUMMARY.

LITHIUM CHLORIDE MEDIUMS FOR PRESERVATION AND RECOVERY OF THE TYPHOID BACILLUS IN FECES. L. C. HAVENS and C. R. MAYFIELD, J. Infect. Dis. 52:157, 1933.

Lithium chloride has a sharp selective action on the typhoid-colon group. In mediums containing concentrations of 0.5 per cent, lactose-fermenting organisms are inhibited, while *Bacillus typhosus* and many of the *Salmonella* group grow well. The advantages of a simple, inorganic compound with uniform action over a complex substance such as brilliant green, which must be titrated and carefully adjusted, are obvious. Mediums containing lithium chloride, both for preservation of feces and for isolation of typhoid bacilli, have been described. The results of comparative tests indicate that acidified bile containing 0.5 per cent lithium chloride,

and 30 per cent glycerin containing this salt, are superior to other preservative mediums. The addition of 0.5 per cent lithium chloride to Endo agar greatly enhances the chances of recovery of small numbers of typhoid bacilli. There is a sharp decrease in the number of colonies of normal fecal bacteria, with a corresponding increase in the number of typhoid colonies. The motility of *B. typhosus* on lithium chloride mediums is usually reduced or entirely lost. Agglutination is of the somatic (O) type, in small, granular clumps. The colonies are smaller, more compact and less translucent. All of these characteristics return to the normal on transfer of the organism to ordinary mediums. Repeated platings from specimens of feces over a period of several days result in a significant increase in the number of positive results. This is particularly true when the preservative medium causes a progressive decrease in the fecal flora originally present.

AUTHORS' SUMMARY.

CATAPHORETIC VELOCITY OF STREPTOCOCCI IN ENCEPHALITIS AND OTHER DISEASES OF THE NERVOUS SYSTEM. E. C. ROSENOW and L. B. JENSEN, *J. Infect. Dis.* **52**:167, 1933.

The results of a study of the cataphoretic velocities of the streptococci isolated from atria of infection of persons suffering from encephalitis and other diseases of the nervous system, and from animals in which characteristic symptoms developed, or which died, following inoculation of the streptococci, are reported. The streptococci as isolated from the nasopharynx and other atria of infection, especially the apexes of pulpless teeth, and sometimes from the blood and involved organs of persons suffering from encephalitis and other diseases of the nervous system, possess a characteristic neurotropic cataphoretic velocity. The velocity of the streptococci as isolated in cases of encephalitis shifted toward the slow velocity of the streptococci from influenza during epidemic waves of influenza. The marked neurotropic type of velocity of the streptococci found during convalescence from influenza suggests, perhaps, why encephalitis and other diseases of the nervous system, such as epidemic hiccup, polio-encephalomyelitis, radiculitis and neuritis, are so prone to occur following attacks of influenza or epidemics of influenza. The serums of almost all patients having encephalitis and certain other diseases of the nervous system had specific velocity-slowness effects on the respective strains of streptococci isolated in cases of encephalitis and having neurotropic velocity. Cataphoretic measurements of the streptococci isolated from atria of infection and measurements of the specific slowing effect of the serum of the patient have proved of value in the differential diagnosis in puzzling cases. Prolonged use of vaccines containing streptococci having chiefly neurotropic velocity, as isolated especially from animals given injections of material derived from patients having encephalitis and other diseases of the nervous system, has been followed by improvement in symptoms and concomitant disappearance of neurotropic streptococci from the nasopharynx in some cases. The results of the studies by the new methods support the hypothesis that streptococci having a common physical characteristic, namely, neurotropic cataphoretic velocity, are directly or indirectly etiologic in the diseases of the nervous system studied.

AUTHORS' SUMMARY.

ANAEROBIC GRAM-NEGATIVE BACILLI FROM ABSCESS OF THE LUNG: TOXIN PRODUCTION AS DEMONSTRATED BY THE SHWARTZMAN PHENOMENON. J. COHEN, *J. Infect. Dis.* **52**:185, 1933.

In this paper there are reported additional observations concerning isolation, cultivation and classification of certain gram-negative anaerobic bacilli isolated from abscess of the lung. For the study of the toxic factors produced by these bacilli, the Shwartzman phenomenon was employed. When the organisms were grown in the culture mediums described in the text and their filtrates injected intravenously into rabbits which had been prepared twenty-four hours previously by cutaneous injection of potent heterologous toxins (i. e., meningococcus toxin),

approximately 80 per cent of the rabbits showed areas of hemorrhagic necrosis. With some organisms from abscess of the lung, attempts were made to reproduce the Shwartzman phenomenon by the use of homologous filtrates for cutaneous preparation. Of these organisms, only filtrates of *Bacterium melaninogenicum* (in symbiosis with *Streptococcus gamma*) and *Leptothrix* proved potent. Further work on the pathogenicity of the various organisms, to be reported, points to the interesting fact that only those organisms which give the positive Shwartzman phenomenon when they are grown in suitable culture mediums are also able to produce severe necrotizing lesions in the lungs of rabbits.

AUTHOR'S SUMMARY.

SELECTIVE ACTION OF CRYSTAL VIOLET AND OF BRILLIANT GREEN ON BACTERIOPHAGES. A. Y. WELLS and N. P. SHERWOOD, J. Infect. Dis. **52**:209, 1933.

A selective phagistatic action of crystal violet and brilliant green on the bacteriophages tested has been demonstrated, and the results suggest that there is a correlation between the selective action of crystal violet on bacteria and their respective bacteriophages. High concentrations of dyes were required to effect inhibition of the bacteriophages. A high dilution of the primary dye solutions (inhibiting dilutions) to the point where there would be no inhibition of bacterial growth was a requisite in demonstrating the activity or inactivity of the lytic principles after each period of incubation. Under the conditions of the experiment, the bacteriophages that lysed gram-positive bacteria were completely inhibited by concentrations of crystal violet which did not appear to diminish the activity of the bacteriophages that lysed gram-negative bacteria. The inhibitive property of brilliant green was extremely variable when compared with that of crystal violet, but nevertheless distinct with one bacteriophage of the colon bacillus in particular.

AUTHORS' SUMMARY.

ACTINOBACILLOSIS OF CATTLE IN THE UNITED STATES. L. THOMPSON, J. Infect. Dis. **52**:223, 1933.

Actinobacillosis is common among cattle in the United States. It would seem that the condition here is similar to that in other countries in which the greater percentage of the so-called bovine actinomycosis is due to *Actinobacillus lignieresii*. The condition known as "wooden tongue" is said by European investigators to be entirely due to this organism. One case of this kind in the present study yielded a culture of *Actinobacillus*. Most cases of involvement only of the cervical glands were due to *Actinobacillus*. On the other hand, the few cases of infection of bone encountered in this series were due to *Actinomyces*. Both *Actinobacillus* and *Actinomyces* produce the aggregations known as "sulphur granules." The quickest way of distinguishing the two organisms in pus is by staining the crushed granules by Gram's method. *Actinomyces* granules stained in this way show numerous gram-positive, rod-shaped forms, whereas those of *Actinobacillus* give no gram-positive organisms, but with careful search a few small, gram-negative, bacillary forms may usually be found. The only points of similarity between the two organisms are the ability to produce similar lesions in cattle and the ability to form sulphur granules. The granules formed by *Actinobacillus* are usually less conspicuous and can often be found only with the aid of the microscope. Agglutinations and absorptions of agglutinins indicate that there is variation among strains of *Actinobacillus* as to antigenic structure. There is, however, a certain amount of cross-agglutination in all strains. Considerable experience with various strains and with control serum would be necessary before the agglutination test could be relied on to detect infection among cattle. It is possible that a better way of preparing antigens might be found.

AUTHOR'S SUMMARY.

THE PATHOLOGY OF PSITTACOSIS IN ANIMALS AND THE DISTRIBUTION OF RICKETTSIA PSITTACI IN THE TISSUES OF MAN AND ANIMALS. R. D. LILLIE, Nat. Inst. Health, Bull. 161, 1933.

The lesions of psittacosis in naturally and experimentally infected parrots and parrakeets are described. *Rickettsia psittaci* has been demonstrated in epithelial cells of the small intestine, ureter, renal secreting tubules and bile ducts, in epithelioid cells, macrophages, reticulo-endothelial cells and mesothelial cells of parrots, in the epithelium of the renal collecting tubules in parrakeets, and in macrophages and alveolar epithelial cells in the lung and in hepatic cells in man. It seems indicated that *Rickettsia psittaci* is primarily an epithelial parasite, and enters cells of the macrophage-reticulo-endothelial series secondarily as the epithelial cells break down. An etiologic relationship of *Rickettsia psittaci* to psittacosis seems indicated, though not proved.

THE LIVER IN A FATAL CASE OF EPIDEMIC "CATARRHAL" JAUNDICE. J. F. GASKELL, J. Path. & Bact. 36:257, 1933.

A case of death from hemorrhage on the third day of acute epidemic "catarrhal" jaundice is described in a patient who had had tonsils and adenoids removed. The condition found was acute hepatitis, the biliary canal system being permeable throughout and intact. The case was not due to spirochetal infection. It is concluded that epidemic "catarrhal" jaundice is a hepatitis. The condition found supports the view that acute and subacute atrophy of the liver are the severe types of epidemic infective jaundice or its sporadic form.

AUTHOR'S SUMMARY.

VARIANTS OF CLOSTRIDIUM WELCHII. C. A. MCGAUGHEY, J. Path. & Bact. 36:263, 1933.

From an authentic strain of *Clostridium welchii*, by selection and repeated subculture of colonies, two variants have been isolated which have remained stable during a period of observation of about four years. The variants, designated 1 and 2, differed from the parent strain and from each other in form of colony, growth in broth, the size and shape of individual bacilli, sporulation and formation of capsules. They resembled the parent strain and each other in fermentation activity, in giving an acrolein test, in liquefying gelatin and in producing a stormy clot in milk. Variant 1 at one stage appeared to be incapable of producing toxin but later produced small amounts of toxin in culture. Variant 2 produced from three to six times as much toxin as the parent strain. The production of hemolysin in culture followed exactly changes in the production of toxin. By agglutination tests the antigens of both variants differed completely from the parent strain. The antigens of variants 1 and 2 were closely related and perhaps identical. Muroid types of the parent strain and of both variants have been noted. The importance of these results in the production of *Cl. welchii* toxin and their possible application to the study of *Cl. welchii* infection and immunity is suggested.

AUTHOR'S SUMMARY.

DISSOCIATION IN CERTAIN MYCOBACTERIA. M. H. CHRISTISON, J. Path. & Bact. 36:285, 1933.

Brinckeroff's "lepra" bacillus, the fish tubercle bacillus (Cobbett), the tortoise tubercle bacillus (Friedmann) and *Mycobacterium rubrum* (Sohnsen 335) dissociate into numerous colony types varying greatly in the complexity of their structure. The colonies show varying degrees of stability which depend to some extent on environmental conditions. There is no evidence of absolute stability among any of the variants, and therefore an attempt to base a classification of these

organisms on colony types and their related characters is regarded as impracticable. Variation also occurs in other characters, such as the type of growth in fluid medium, emulsibility in saline solution, cell morphology, pigment formation and, in the case of the fish tubercle bacillus, ability to absorb dye from gentian violet-egg medium.

AUTHOR'S SUMMARY.

PNEUMOCOCCAL INFECTION IN INFANCY AND CHILDHOOD. J. W. S. BLACKLOCK and K. J. GUTHRIE, *J. Path. & Bact.* **36**:349, 1933.

The pathology and bacteriology of pneumococcic infections in infants and children of the hospital class are discussed. In cases of acute respiratory disease, throat swabs were used for isolating the pneumococcus as sputum is not easily obtained in the young child. In 38.6 per cent of healthy children, pneumococci were found in the throat, 96.7 per cent of these being group IV strains. The accuracy of the results of the examination of the throat swab in a series of cases of pneumonia were confirmed in 88.5 per cent. Some of the cases of pneumonia which did not come to autopsy could not be definitely classified. In a few cases the lesions in the lungs histologically showed characters of both lobar pneumonia and bronchopneumonia; such mixed types were most frequent in the second year of life. Pneumococci from 140 cases of pneumonia were typed, 9.3 per cent belonging to the fixed types (chiefly types I and II) and 90.7 per cent to group IV. The mortality rate of the fixed type infections was 38.5 per cent and of the group IV infections, 40.9 per cent. In adult cases of lobar pneumonia investigated simultaneously in the same city, 73 per cent were due to types I, II and III pneumococci and only 27 per cent to group IV. The bacteriology of bronchopneumonia was studied post mortem in a series of cases. Pneumococci were found in 85.5 per cent, streptococci in 7.3 per cent, influenza bacilli in 6.1 per cent and *Staphylococcus aureus* in 1.1 per cent. Pus from the pleura in 114 cases of empyema yielded pneumococci in 68.4 per cent, streptococci in 17.5 per cent and other organisms in 14 per cent. The mortality was slightly higher in pneumococcic than in streptococcic empyemas. Pneumococci were typed in 66 cases of empyema, and 30 belonged to type I, 1 to type II, and 35 to group IV. The incidence of fixed type infections rose with the age of the child, group IV strains predominating in the empyemas following bronchopneumonia in early childhood. The mortality rate in fixed type infections was 32.2 per cent, and in group IV infections, 54.3 per cent. Of 27 cases of primary pneumococcic peritonitis, 24 (88.9 per cent) were in girls and 3 in boys. In 10 of the 11 female children examined at autopsy, the peritoneal lesion was most acute in the pelvis. Of the 3 boys, only 1 was examined post mortem, when no lesion except peritonitis was found. In 13 cases type I, and in 2 cases type II, pneumococci were isolated from the peritoneal exudate. Pneumococcic peritonitis secondary to pneumonia was rare, occurring in only 2.4 per cent of the cases, and in these the thoracic lesions were severe. Pneumococcic meningitis occurred most frequently as a sequel to acute otitis media and was primary in only 17.6 per cent of the cases studied. Of the pneumococci isolated from the cerebrospinal fluid, 84.2 per cent belonged to group IV. Purulent otitis media was present in 21.1 per cent of 2,000 consecutive autopsies; the pneumococcus was the predominating organism in the pus from this lesion. The commonest complication in a series of cases of pneumonia examined post mortem was acute otitis media. In 33 cases of acute middle ear disease, pneumococci were typed, 93.9 per cent being group IV strains. Infection was due to group IV pneumococci in 70.6 per cent of a series of surgical lesions, (arthritis, orchitis and acute abscesses). On arranging the 292 strains of pneumococci isolated from various acute lesions according to type of coccus and age of patient, it was found that the percentage of infections with fixed types was much greater in older children than in those under 3 years.

AUTHORS' SUMMARY.



DIPHTHERIA-LIKE ORGANISMS FROM NASOPHARYNX. M. M. BARRATT, J. Path. & Bact. **36**:369, 1933.

A group of aberrant diphtheria-like organisms have been described which, while resembling *Corynebacterium diphtheriae*, differ from it in certain cultural characters, most notably in their capacity to liquefy gelatin. The lesions produced in guinea-pigs by intradermal injection of the organisms are more papular and pustular than those following injection of typical *C. diphtheriae*. Diphtheria antitoxin does not prevent these cutaneous lesions and even in large doses does not save the life of the animal if more than one or two minimal lethal doses are given subcutaneously or with some strains even if only one is given. Toxic filtrates produce lesions of the skin which do not resemble those of *C. diphtheriae*. Diphtheria antitoxin, unless given in larger doses than from 80 to 100 units to one minimum lethal dose of toxin, does not protect the animal from death. The aberrant strains differ further from *C. diphtheriae* in being pathogenic for rats by intraperitoneal inoculation. When the characters of the aberrant strains differ culturally or biologically from those of *C. diphtheriae*, they approach, if not completely, those of *Corynebacterium pseudotuberculosis-ovis* (bacillus of Preisz-Nocard). Although evidence is lacking of the association of the strains studied with severe cases of clinical diphtheria, the occurrence in the human nasopharynx of diphtheria-like organisms, the pathogenic effects of which in the guinea-pig are not prevented by diphtheria antitoxin, has been established. Therefore, the possibility that strains of this type play a part in the causation of cases which do not respond favorably to antitoxin cannot be altogether ignored.

AUTHOR'S SUMMARY.

QUANTITATIVE DETERMINATION OF BACTERIOPHAGE ACTIVITY. G. DREYER and M. L. CAMPBELL-RENTON, J. Path. & Bact. **36**:399, 1933.

Evidence has been produced to show that the generally accepted view that the number of plaques produced increases in direct proportion to the increase in the concentration of the bacteriophage does not hold, but that a relatively smaller number of plaques is produced by the higher concentration. The ratio between the concentration of the bacteriophage and the number of plaques formed may be represented graphically by a "standard" curve, based on our observations. This standard curve is applicable to all the experiments we have carried out, independent of the strength of the bacteriophage, the number of bacteria on which it acts and the concentration of agar. It also seems to apply to results obtained by some previous workers. This standard curve constitutes a basis and fixed standard for the quantitative determination of the activity of any bacteriophage. We have shown, in agreement with Gratia, Gjörup and Burnet, and contrary to the observations of d'Herelle, Bronfenbrenner and Korb, Asheshov, Marshall and others, that the number of plaques produced by the same concentration of bacteriophage increases with increasing numbers of bacteria, up to a maximum, and then tends to decrease; at the same time the size of the plaques is diminished. When bacteriophage acts on bacteria spread on 1.5 and 4 per cent agar, respectively, fewer and smaller plaques are produced by the same concentration of bacteriophage on the strong agar than on the weak agar. The admixture of homologous organisms killed at 100 C. to the inoculum reduces the activity of the bacteriophage. It has been found that the Pasteur bacteriophage used for our experiments deteriorates rapidly at 37 C., and that the rate of deterioration may be satisfactorily expressed by the equation for a monomolecular reaction.

AUTHORS' SUMMARY.

THE APPENDIX IN THE PRODROMAL STAGE OF MEASLES. W. H. SCHULTZE, München. med. Wchnschr. **80**:576, 1933.

Histologic sections of an appendix removed from a girl 10 years of age, with appendical symptoms, showed no evidence of acute appendicitis, but did show large numbers of giant cells in the mucosa and submucosa. Ten or more nuclei were present in the cells. Schultze was familiar with the findings of similar giant cells

in the appendix during the prodromal stage of measles which were reported by Finkeldey (Finkeldey, W.: *Virchows Arch. f. path. Anat.* 284:518, 1932) and by Walter Fischer at Wiesbaden in 1932. Schultze diagnosed measles from the histologic picture, and a typical rash actually developed in the child on the day following the operation. Schultze was apparently unaware of the fact that the first case of this type was reported in the American literature by Herzberg in January, 1932 (*J. A. M. A.* 98:139, 1932).

I. DAVIDSOHN.

## Immunology

TUBERCULOUS SKIN REACTION. E. S. MARIETTE and E. P. K. FENGER, *Am. Rev. Tuberc.* 25:357, 1932.

The M A 100 human protein (identical with the original tuberculo-protein isolated by Seibert) is as sensitive and as selective as old tuberculin, and probably more so. The initial and subsequent doses recommended for the M A 100 human protein are safe, in that dangerous reactions are not encountered. The initial and subsequent doses recommended for M A 100 human protein are large enough to identify the majority of tuberculous persons, and apparently do not need to be increased. The M A 100 proteins are apparently not specific, at least in large doses. There is apparently a protein substance common to all acid-fast bacilli, which if given in large enough doses, will elicit the same type of reaction as that obtained from old tuberculin. As the M A 100 protein represents a substance in a purified form which can always be reproduced at the same iso-electric point and which can be weighed out in milligram doses, so that the exact content of the solution in milligrams is known, it is a better testing substance than old tuberculin

H. J. CORPER.

SERUM SICKNESS IN RABBITS. M. S. FLEISHER and L. JONES, *J. Immunol.* 24:369 and 383, 1933.

The serum of individual normal horses shows variations in activity in producing serum sickness in rabbits, so that the occurrence of this manifestation may vary between 93 and 24 per cent. Similar variations in the activity of two batches of pooled serums have been noted, the variations being between 60 and 35 per cent. Differences have also been noted in the activity of antisera of various types. No evidence has been obtained suggesting that the immunization of animals increases or materially alters in any way the possibility of the serum causing serum sickness. It appears that beef serum is probably less active in causing serum sickness than is horse serum; and sheep or hog serum is definitely less active than horse or beef serum in this regard. The addition of as much as a 0.35 per cent concentration of phenol to serum does not apparently alter its activity in causing serum sickness. Aging of the serum (up to nineteen months) does not alter its power to cause serum sickness. The reactivity of the individual animal may be a determining factor in the occurrence of serum sickness.

When rabbits receive a second injection of serum after an interval of time, they may show reactions of immediate, accelerated or normal serum sickness. These reactions in the rabbits are essentially analogous to the reactions appearing in man subsequent to a second injection of serum. The time elapsing between the first and second injections appears to be a factor in determining the type of reaction which will occur. If only twelve days elapse, no abnormal reactions are noted; if about two weeks, a few mild accelerated reactions appear; and if from twenty to thirty-three days, a larger number of more marked accelerated reactions are evident, and a few immediate reactions occur. At from thirty-six to fifty-three days more immediate reactions and quite a number of accelerated reactions occur; at this period abnormal reactions occur in a larger percentage of animals than at any other. At from three to four and one-half months only immediate reactions were observed. Finally, at six months no immediate reactions were produced; a few accelerated reactions were noted, and a number of reactions which were on the borderline between accelerated and normal and a number of normal reactions were observed.

AUTHORS' SUMMARIES.

HEMORRHAGIC ALLEGRY: II. SHWARTZMAN AND ARTHUS PHENOMENA. ANDRÉ GRATIA and ROGER LINZ, *Ann. Inst. Pasteur* 50:89, 1933.

In rabbits given 0.5 cc. of horse serum intravenously followed after eight weeks by 0.1 cc. injected intradermally the edema of the Arthus phenomenon developed. As the test was repeated more congestion and less edema were apparent, and the lesion finally appeared as an intense hemorrhagic lesion identical with Shwartzman's reaction and not given in the classic descriptions of the Arthus phenomenon. From about 15 to 20 per cent of the experiments performed on rabbits yielded in a marked degree the result described, and as many animals were refractory—a distribution correlating with the Shwartzman reaction, and including identical animals. Fatal anaphylactic shock occurred in the animals with the hemorrhagic lesions, but not in the refractory animals. Both the Arthus and Shwartzman reactions occurred in the same animal simultaneously, appearing eventually identical and without reciprocal desensitization. Each reaction, however, failed to desensitize specifically against its own type. Both reactions desensitized guinea-pigs and rabbits against serum shocks, often only partially. Reciprocally, a nonfatal serum shock desensitized these animals against the Arthus reaction but apparently not against the more violent Shwartzman reaction. There appears to be only a simple quantitative difference in intensity and rapidity between these reactions, as though microbial products stimulated a local hypersensitivity in a few hours, whereas other antigens required several weeks.

FROM AUTHORS' CONCLUSIONS.

#### GRUNDFRAGEN DER IMMUNBIOLOGIE UND ALLERGIELEHRE.

This is a special number of *Immunität, Allergie und Infektionskrankheiten*. The editor-in-chief is Fr. Michélssohn, and among the associate editors are Degkwitz, Schlossberger, van Leeuwen and others. The publisher is Otto Gmelin, Munich. The journal serves the needs of the practicing physician; its purpose is therefore informatory. The contents are reviews by authors who are recognized authorities on their assigned subjects. In the special issue which is reviewed nine authors discuss on 112 pages subjects well deserving to be summarized, as is done in the titles. The original contributions of the authors form the background against which the results obtained by other authors are projected and discussed. L. Bogen-dorfer discusses the relation between immunity and the reticulo-endothelial and endocrine systems. In addition to others, his own experiments have established such relations, as is shown by the fact that severing the spinal cord at the level of the sixth cervical vertebra inhibited the development of agglutinins in dogs. The same inhibition was brought about by the administration of a sympathicotropic drug (ergotamine). E. F. Mueller writes about the skin, the sympathetic nervous system and susceptibility to disease. Ivanic and Dimitrijevic-Speth summarize the biokinetic theory of infection, the experimental basis of which is largely their own contribution. The motility of bacteria is linked with their pathogenicity and virulence in a very convincing manner. A new classification of infectious diseases is deduced. Chapters on latent infection, by Schlossberger and Koch, and on parodontal focal infection, by Leschke, follow. Pockels reviews his successful attempts at increasing immunologic responses by the administration of extracts of the spleen and the lungs and of reticular endothelium. Niekerk reviews the present knowledge of allergens and brings out the weaknesses of the hypothesis of the all-important significance of heredity in allergic susceptibility. It is possible that the differences between allergic and normal persons are merely quantitative. Recent investigations on the chemistry of allergens are analyzed. A great deal of information is condensed in 13 pages on allergic diathesis by Kaemmerer. He defines diathesis as an individual, congenital and, frequently, hereditary condition, in which physiologic stimuli produce an abnormal reaction. He advocates the use of the term "diathesis" in preference to the terms "constitution" and "disposition" because the former has the meaning of a nonspecific state and the latter of a transient state. Kaemmerer agrees with the great number of those who are unable to see an absolute difference between anaphylactic conditions in animals and the phenomena of allergic hypersensitivity in man. In the final chapter Hofbauer stresses the significance in asthma of local conditions in the upper respiratory

tract, particularly of the nose, and supports his contentions by means of well selected experiments on animals. In addition to the local conditions, internal secretory disturbances are pathogenic factors.

I. DAVIDSOHN.

DEFENSE MECHANISMS IN CHICKEN SPIROCHETOSIS. I. L. KRITSCHESKI and P. L. RUBINSTEIN, *Virchows Arch. f. path. Anat.* **287**:566, 1932.

Chickens infected with *Spirochaeta gallinarum* were killed at different intervals. Histologic examination of the organs with special spirochetal stains revealed that the spirochetes gradually become dissolved after undergoing degenerative changes such as splitting and fragmentation. Phagocytic processes were seen only rarely and apparently do not play an essential rôle in the destruction of the spirochetes. It seems most likely, therefore, that the destruction of the spirochetes is due to the action of specific lysins.

W. SAPHIR.

ISOLATION OF GROUP-SPECIFIC AGGLUTINOGENS OF ERYTHROCYTES. A. JUHÁSZ-SCHÄFFER and A. VANNOTH, *Ztschr. f. d. ges. exper. Med.* **86**:809, 1933.

The authors have attempted to separate the agglutinin factor from the stroma of erythrocytes. This problem is of practical importance for the determination of questionable blood groups. Previous authors (Hektoen, Schulhof and Jacoby) have shown that this factor is of albuminous nature. The agglutinin is bound to the stroma of the erythrocyte and cannot be demonstrated in the serum. The stroma forms agglutinin even after removal of the hemoglobin. The authors were unable to demonstrate an agglutininogen by washing erythrocytes in a physiologic solution of sodium chloride or by causing hemolysis. Nor were they able to inhibit agglutination by these means. It is concluded that the agglutinin principle of the erythrocytes cannot be separated from the stroma. It seems that iso-agglutination is due to a physicochemical property of the stroma rather than to a special substance.

L. KLEIN.

## Tumors

PRODUCTION OF SARCOMA BY BACILLI. V. FISCHL and E. KUSSAT, *Ztschr. f. Krebsforsch.* **36**:276, 1932.

The experimental results reported earlier in 1932 by Bellows and Askanazy, to the effect that spore-forming bacilli isolated from ripe tomatoes would induce peritoneal sarcomatosis of rats if injected repeatedly, could not be confirmed by these writers. Organisms from other vegetable sources were similarly tested without result.

H. E. EGGERS.

FREQUENCY OF PULMONARY CANCER. A. E. SITSEN, *Ztschr. f. Krebsforsch.* **36**:313, 1932.

Sitsen questions the authenticity of the statistics that have appeared with some frequency tending to show an increase of cancer of the lung in recent years. He points out that with them attention is seldom paid to the altered age distribution in the general population, and he states that no increase in the incidence of this cancer could be found in statistics collected by him at Innsbruck, after this factor had been evaluated.

H. E. EGGERS.

MALIGNANT TUMORS AND ARTERIOSCLEROSIS. J. CASPER, *Ztschr. f. Krebsforsch.* **36**:354, 1932.

Casper presents statistical evidence from his own collection that as a rule cancer and arteriosclerosis do not occur in association, though, of course, the rule is one with numerous exceptions. This negative relationship can scarcely be a matter of direct causal association, and Casper suggests that the explanation may be a matter of cholesterol metabolism. Animal experimentation, however, would

tend to show that an excess of cholesterol contributes toward both arteriosclerosis and the occurrence of cancer, so that the explanation, if along these lines, must rest on some as yet unrecognized alteration of cholesterol metabolism.

H. E. EGGERS.

INCREASED INCIDENCE OF PULMONARY CANCER. A. SYREK, *Ztschr. f. Krebsforsch.* **36**:409, 1932.

In autopsy statistics collected by the Gesellschaft der Ärzte at Warsaw, there has been a threefold increase in cancer of the lung from 1925 to 1930 over the corresponding period from 1919 to 1924. As usual, the cases are predominantly in male patients. The Warsaw statistics are of some especial interest, as they apparently rule out certain suggested etiologic factors. Industrial irritation may apparently be excluded, as the cases were largely of rural origin; so, too, the possible effect of automobile exhaust gases. The predominance in males would cast doubt on the effect of previous influenza. Of other suggested factors, the effects of tobacco and of exposure to war gases remain as possibilities.

H. E. EGGERS.

CARCINOSARCOMA. M. BÖSENBERG, *Ztschr. f. Krebsforsch.* **36**:416, 1932.

Bösenberg reports six cases of what he considers mixed malignant growths. The first is a squamous cell carcinoma in association with a spindle cell sarcoma; the second, an adenocarcinoma and a mixed cell sarcoma of the liver; the third, a keratinizing prickle cell carcinoma and a mixed cell sarcoma; the fourth, a similar carcinoma with a lymphosarcoma (?) of the uterus; the fifth, a slightly keratinized prickle cell carcinoma and a mixed cell sarcoma of the lung, and the sixth, a basal cell carcinoma and a spindle cell sarcoma of the skin. The author considers the last two cases as collision tumors. A rather extensive bibliography is appended.

H. E. EGGERS.

FREQUENCY OF BRONCHIAL AND PULMONARY CANCERS IN 1925-1931. E. DISEMANN, *Ztschr. f. Krebsforsch.* **36**:563, 1932.

While the yearly incidence of pulmonary cancer as observed at the Deutsche pathologische Institut at Prague during the years considered showed a marked fluctuation at the end of the period the deaths from these cancers were found to constitute 16.47 per cent of all deaths from carcinoma. Eighty of 7,855 autopsies, or 1.02 per cent, revealed bronchial or pulmonary cancers; that is, these types constituted 10.85 per cent of all cases of cancer. This was a definite increase over the preceding five years. In regard to the distribution according to age, the highest incidence was seen in the sixth decade. The increase involved principally the male sex, in which the rate was quadruple that of the preceding five years, while in female patients it was double. A considerable proportion of the patients had been unduly exposed to excessive inhalation of smoke or dust. More than 50 per cent came to Prague from western Bohemia.

H. E. EGGERS.

CYTODIAGNOSIS OF MALIGNANCY IN PUNCTURE FLUIDS AND SECRETIONS. H. KARP, *Ztschr. f. Krebsforsch.* **36**:579, 1932.

Karp has made a critical study of the method of cytodiagnosis of malignant tumors as suggested by Quensel, and emphasizes the following differential criteria between cells desquamated from serous linings and those of cancerous growth: The former cells are ordinarily from 15 to 20 microns in diameter, are regularly round or ovoid in outline, and frequently are grouped in irregular plaques, the margins of which are obscured by a sheath of structureless proteid material. Fat deposits or vacuoles are present in the cytoplasm with some frequency; the nuclei range from 5 to 12 microns in diameter, and the nucleoli, from 1 to 2 microns. The ratio of nucleolar to nuclear surface varies from 1:25 to 1:100.

With tumor cells, there is much greater variation of size; vacuolation may be characteristic, with the appearance of peculiar giant vacuoles from 40 or 50 microns in diameter to a maximum of 90 microns. Nuclear diameters range from 5 to 24 microns; those of the nucleoli, from 4 to 12 microns. The relative increase of the nucleolus is almost characteristic, and the ratio of areas may range from 1:20 to 1:4. Irregular deposition of chromatin—pyknosis or local accumulation—is more frequent in tumor cells, and hyperchromatosis of the nuclear margin is frequently present. Either type of cell may show phagocytosis. With these criteria, diagnoses have been confirmed at autopsy with some regularity.

H. E. EGGERS.

PRIMARY MALIGNANT TUMORS OF SEROUS LININGS. B. FISCHER-WASELS. *Ztschr. f. Krebsforsch.* **37**:21, 1932.

In the opinion of Fischer-Wasels, the existence of tumors, either benign or malignant, of primary origin in the cells lining the serous body cavities is exceedingly doubtful. Definite demonstration of such origin has still to be made. In tumors which have up to the present been reported as of this character, nothing convincing has been observed. In general, their properties are those of other common tumor forms, usually those of undifferentiated carcinomas. Tumors of unquestionable primary location in these sites are regarded as originating from the results of embryologic displacement. In the author's opinion, the identification of this group of tumors by the demonstration of histologically intermediate cell types is unconvincing and productive of error.

H. E. EGGERS.

INCREASE OF PULMONARY CANCER AND ITS PATHOGENESIS. W. NOWICKI. *Ztschr. f. Krebsforsch.* **37**:83, 1932.

This study of the relative incidence of pulmonary cancer is based on autopsy material observed at Lwow (Poland) during the last thirty-five years, amounting to approximately 31,000 cases. Of the seven five year periods considered, in the first the incidence of cancer of the lung amounted to 0.07 per cent. From 1916 to 1920, it constituted 0.60 per cent; from 1921 to 1925, 0.38 per cent, and from 1926 to 1930, 0.47 per cent. The corresponding figures as related to total cases of cancer are 1.8, 9.3, 5.3 and 7.2 per cent. The increase is most striking when the last fifteen year period is compared with the similar one preceding; this shows more than doubling of the incidence. Three fourths of the cases occurred in male patients; Jews and Gentiles were affected alike; more than 50 per cent of the cases occurred in persons between the ages of 40 and 60. Tuberculosis was associated only rarely. No definite etiologic factor could be recognized, and Nowicki conjectures that chronic bronchial irritation, by the production of metaplastic changes of the bronchial epithelium and the tendency of such changes to become cancerous, may be of indirect importance.

H. E. EGGERS.

DIAGNOSIS OF CANCER BY PHOTOMETRIC DETERMINATIONS IN VENOUS BLOOD. E. SEHRT. *Ztschr. f. Krebsforsch.* **37**:94, 1932.

Basing his work on the theory that cancer is associated with general and fundamental changes in body oxidation, with involvement of the hemoglobin, Sehrt describes the diagnostic reactions based on the altered behavior of the hemoglobin with respect to oxidative changes. In the first reaction reported, the differential behavior of venous blood in response to *a*-naphthol and dimethylparaphenylenediamine, with and without the addition of potassium ferricyanide, is noted. The blue colors developed under these circumstances are compared; in normal blood, the difference between them is approximately twice that shown in the presence of cancer. In the second reaction, the basis is the behavior of the hemoglobin in response to reduced methylene blue (methylthionine chloride, U. S. P.); in cases of cancer there is a definite increase of the oxidizing power of the hemoglobin. Sehrt believes that the increased and relatively stable oxygen-combining power of the hemoglobin is at least in part responsible for diminished tissue oxidation, and

he regards this as an important contributing factor in the genesis of cancer. In general, his results were well substantiated by the later course of the cases studied; however, among his normal controls, 12 per cent gave reactions of the cancerous type to his first test, and he regards this as evidence of a cancerous predisposition in these cases. Persons with esophageal cancers gave negative reactions to both tests.

H. E. EGGERS.

CANCERIGENIC PRINCIPLE OF OILS AND FATS. T. GASSMANN, *Ztschr. f. Krebsforsch.* **37**:117, 1932.

On the analysis of extracts of crude coal tar and tobacco ash, Gassmann found a phosphorus-iron compound to which he assigned the formula  $\text{Fe}(\text{P}_2\text{O})_2$ . He believes that this is the active cancerigenic agent of these substances. He found it, in combination with formic acid, in the urine of patients with cancer.

H. E. EGGERS.

CANCER OF THE FEMALE GENITALIA IN CHILDREN. K. K. ORTMANN, *Ztschr. f. Krebsforsch.* **37**:283, 1932.

The author reports a case of vaginal cancer in a child  $1\frac{1}{2}$  years of age. Morphologically, the cancer was of an undifferentiated type; the photomicrographs suggest a similarity to the less differentiated squamous cell carcinomas, but it is stated that there were occasional suggestions of tubule formation. Two other cases of vaginal cancer and six of uterine carcinoma occurring in children are cited.

H. E. EGGERS.

ATYPICAL EPITHELIUM AND MALIGNANT NEW GROWTHS. H. T. DEELMAN, *Ztschr. f. Krebsforsch.* **37**:374, 1932.

Deelman presents a critical study of a number of non-neoplastic atypical epithelial new growths, with the considerations that led to their diagnoses. In the last case, he is forced to acknowledge that the history was an absolutely essential item in the diagnosis. This case was one of syphilitic hypertrophy of the skin. It illustrates strikingly the close resemblance of some of these lesions to cancer of the skin, a resemblance which has worried numerous pathologists.

H. E. EGGERS.

RELATIONS BETWEEN BENIGN AND MALIGNANT NEW GROWTHS. H. T. DEELMAN, *Ztschr. f. Krebsforsch.* **37**:383, 1932.

The study of experimental tar cancers has shown that with them there are stages in which the character of the lesion as regards malignancy cannot be determined on morphologic evidence. Similarly, there are human lesions which likewise are of uncertain character, and which, being biologically malignant, may fail to betray the fact in their morphology. The so-called transition from a benign to a malignant lesion, in Deelman's opinion, really signifies that morphologic evidence of malignancy has not yet appeared in lesions which are already biologically cancerous.

H. E. EGGERS.

CALCIFIED EPITHELIOMA OF THE SKIN. I. TANASESCU and N. P. BALAN, *Ztschr. f. Krebsforsch.* **37**:398, 1932.

The case reported occurred in a child of 8, who had double lesions of apparently infiltrative overgrowth of the skin, with extensive calcification of the more deeply seated epithelial colonies. The infiltration was not malignant in the clinical sense. The authors explain the recurrence after operation as the result of unremoved accessory nodules. A review of similar cases is included.

H. E. EGGERS.

INSULAR CARCINOMA OF THE PANCREAS. H. HAMDI, *Ztschr. f. Krebsforsch.* **37**: 411, 1932.

In the case reported the primary tumor was seated in the tail of the pancreas, with free infiltration throughout the remainder of the organ and numerous metastases in the liver. The diagnosis was made on morphologic grounds alone, and no mention is made of the aberration of sugar metabolism which is usually such a prominent feature of carcinoma of the islands of Langerhans.

H. E. EGGERS.

MALIGNANT TUMORS PRODUCED BY TOMATO JUICE. M. PLONSKIER, *Ztschr. f. Krebsforsch.* **37**:492, 1932.

Plonskier's experimental observations constitute one of the few exceptions to the negative results obtained by almost all investigators who have attempted to reproduce Bellow's reported induction of sarcoma in rats by the injection of tomato juice. In two of six rats given five injections of tomato juice he observed the appearance of sarcoma, a diagnosis which was confirmed not only by the morphology of these tumors, but also by the presence of extensive metastases in the spleen, liver and lymph glands. However, the tumors were not transferable by inoculation.

H. E. EGGERS.

EXPERIMENTAL PRODUCTION OF SARCOMA IN RATS. G. KLEIN, J. KLINKE and R. HANSER, *Ztschr. f. Krebsforsch.* **37**:539, 1932.

These writers have attempted unsuccessfully to repeat Bellow's experiments on the induction of sarcoma in rats by the injection of tomato juice or of organisms obtained from it. When intact, not overripe tomatoes were used, bacterial infection appeared only when the skin had been damaged. With fruit of this character, among other organisms bacteria resembling *Bacillus subtilis* were recovered. But the intraperitoneal injection of these, as well as of filtrates and ultrafiltrates of tomato juice, failed to cause other than granulomatous growths; similar growths were obtained by the injection of the juices of apples and grapes, and their appearance suggested an origin in mechanical irritation. Their morphology, however, suggested the possibility of later sarcomatous change.

H. E. EGGERS.

CANCER OF THE LUNG. W. PETERS, *Ztschr. f. Krebsforsch.* **37**:587, 1932.

Peters studied the incidence of cancer of the lung from material gathered at the Moabite Krankenhaus in Berlin. The increase has been definite, from the fifth most frequent form of cancer, which was its status in 1905-1906, to the second most frequent in 1925-1926. The increase was principally in the male sex; in 1905-1906 to sex ratio was 3:1, and 1925-1926, 6:1. Cancer of other organs does not show a corresponding increase, and gastric cancer has even diminished in frequency. Statistics from other sources, compiled and compared similarly, as a rule show a similar increase, but there are exceptions, occasionally found in only one of two nearby communities, a phenomenon without explanation. Four cases of cancer of the lung are described; one of them originated in the wall of a tuberculous cavity.

H. E. EGGERS.



# Society Transactions

## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, Oct. 9, 1933*

E. H. HATTON, *President, in the Chair*

THE ORIGIN AND PATHOLOGIC SIGNIFICANCE OF THE EPITHELIUM FOUND ABOUT THE ROOTS OF TEETH: PRESIDENTIAL ADDRESS. E. H. HATTON.

Malassez (Arch. de physiol. norm. et path. 5:129, 1885) is generally credited with the first description of the epithelial bodies found about the roots of teeth:

"The development of pure epithelial tumors within the jaw, which are remote from known epithelial structures; the resemblance of certain parts of these tumors to different types of epithelium which are concerned in the formation of teeth or which accompany it, and the existence of small cellular bodies in the normal gums of the adult that likewise resemble the same have long since brought me to the belief that in the jaws of adults there must be found epithelial rests originating from the formation of teeth.

"This hypothesis, which is so attractive and probable in its conception demands anatomic confirmation. The result of my work has been the disclosure of small cell nests about the roots of the teeth of adults, and in normal relationships, masses which must be considered as epithelial rests of the tooth-forming process and as the origin of certain intramaxillary new growths."

Malassez examined one lower jaw by histologic methods to supply the evidence on which this report was based. The studies were made on two incisors, one cuspid, two premolars and one molar. He found in most of the sections that: "in truth, small cellular masses existed, which had their location for the most part in the innermost part of the alveolodental ligament close to the substance of the tooth, and, indeed, often almost in contact with the cementum. However, I have found them, less seldom, in the outermost portion adjacent to the bone and, in isolated instances, even in medullary spaces within the contiguous bone. . . . The deepest are found about the extremity of the root; the most superficial are located just beneath the gingival attachment to the tooth. It is these superficial portions that I had previously observed in the gums and that Serres had considered as dental calculus glands.

"Is the presence of these epithelial rests constant, frequent or exceptional? I am not able to answer this question exactly; all that I can say is that I have found them about all the teeth that I have examined. . . . What name shall be given to these epithelial masses, which, so far as I know, have not been described? Because of their origin and nature and in accordance with present terminology, I propose the name *amas épithéliaux parodontaire* (periodontal epithelial débris)."

In succeeding sections of the monograph based on these studies and in other monographs Malassez attempted not only to defend his conception of the origin of the periodontal epithelial débris but also to relate it to twelve conditions, among which are: radicular dental fungosities, radicular dental cysts, intramaxillary epithelial tumors, multilocular cysts of the lower jaw, odontoblastic cysts, odontomas and carcinomas. The suspicion remains that he arrived at his conclusions largely by deductive processes rather than by carefully controlled experimentation and extensive laboratory study. On the other hand, his descriptions and illustrations of the material studied do not deviate in any material respect from those of the most recent investigators.

A great deal of unnecessary confusion has arisen concerning the significance of the work of Malassez, because a French anatomist, Serres (*Essai sur l'anatomie et la physiologie des dents, ou nouvelle théorie de la dentition*, Paris, Mequignon-Marvis, 1817) described epithelial structures in the gums under the name of tartar glands, which are considered by many to be identical with the epithelial débris of Malassez. Malassez, however, was familiar with the work of Serres and insisted that his epithelial débris was entirely distinct in origin and significance from the structures studied by Serres, which are described in the following words:

"The dental glands, because of their minuteness, have hitherto eluded the researches of anatomists. Nevertheless, the gums of the fetus at term contain a considerable quantity. Their function at this time appears to be the lubrication of the cartilages which serve for suction when retaining the nipple of the mother.

"I was led to the discovery of these small glandular bodies in looking for the opening of the gubernaculum dentis on the jaws of a fetus at term. I found four or five whitish bodies side by side; when they were pressed firmly, after a small opening had been made, a white substance of the consistency of wax and assuming a spiral form exuded from them. On examining the two jaws attentively, I found a multitude of similar glands distributed throughout the cartilaginous substance which at this time forms the gums. . . . I detached many of these bodies, the size of which equalled that of a millet seed and which were similar to the meibomian glands. I could not discover any distinct opening; the microscope showed only a small brown point in the middle; the white substance contained in the interior did not exude unless an opening was made in the little sac. These glands, then, appear to be formed of a little sac or cyst secreting and containing this white matter and allowing it to transude by their pores or by the little black point which the microscope revealed. The largest are situated on the inner side of the gums in the kind of groove found in this situation. These glands, as we have previously stated, serve to lubricate the cartilages which in the fetus take the place of the teeth before their appearance, but after the eruption of the teeth they secrete the substance which is known as tartar."

It must be clear from this description that the structures described by Serres are very superficial and have nothing to do with the dental follicle. Further discussion of their character and significance is irrelevant here.

Malassez' contention that his epithelial débris originates from the disintegration of the epithelial structures of the tooth germ was challenged at home and abroad. Magitot and LeGros (*J. l'anat. et physiol.* **15**:286, 1879) asserted that as soon as the function of the enamel organ had been accomplished it must atrophy and disappear. Von Kölliker (*Handbuch der Gewebelehre des Menschen*, Leipzig, Wilhelm Engelmann, 1867, p. 494) believed the same. Others, though granting the existence of the epithelial débris, argued that it had nothing to do with the origin of dental root cysts and other pathologic entities listed by Malassez. Of these, perhaps Grawitz is the best known, although his report appeared much later and is reserved for later mention.

Dependable observations accumulated slowly as embryologists and histologists gradually untangled the process of tooth development. Von Brunn (*Arch. f. mikr. Anat.* **29**:367, 1887) supplied an excellent description of the enamel organ and emphasized that the epithelium not only covers the enamel cap but also extends the full length of the root, enclosing it in a sheath. He insisted that the function of the enamel organ is to give form to the tooth, and that the formation of enamel is merely an accessory function. He said: "Its presence is evidently necessary for the odontoblast being properly placed, namely, against its inner surface; it forms the matrix for the later dentine substance. When the odontoblasts occupy the proper position, when the first layer of dentine has been formed and in this way the shape of the tooth has been secured, the epithelium of the enamel has played its part for that portion of the tooth." Hertwig (*Die Elemente der Entwicklungslehre des Menschen und der Wirbelthiere*, Jena, Gustav Fischer, 1900) made similar observations, and his name has been attached to the epithelial sheath that surrounds the developing tooth. (An excellent discussion

of this subject has been supplied by Dr. Kaethe W. Dewey [in Moorehead, F. B., and Dewey, K. W.: *Pathology of the Mouth*, Philadelphia, W. B. Saunders Company, 1925, p. 432].)

It has been assumed that this sheath remains intact until the connective tissue invades it in order to produce the cementum covering of the root, and that at this time its disintegration and atrophy are associated with the formation of the epithelial débris of the periodontal membrane. In this conception the enamel portion of the tooth was considered relatively fixed, and the root with its attendant sheath was thought to grow inward. This classic conception has been challenged by at least one school of histologists, who stated that Hertwig's sheath does not grow inward but that the loop constitutes a relatively fixed point from which the root portion grows outward, carrying the crown with it. Even during the developmental period the sheath is continuous only at the loop portion near the apex, and as the connective tissue portions of the tooth and follicle grow faster than the epithelium, disintegration begins early. However, the sheath plays an important part in the development of the bifurcation of the multirooted teeth, and larger masses of epithelium are deposited at these bifurcations (Orbán, B.: *Dental Histology and Embryology*, ed. 2, New York, P. Blakiston's Son & Co., 1929, p. 129). This may have some pathologic significance, as will be shown later.

The existence of these epithelial bodies in the periodontal membrane, substantially as described by Malassez, is generally accepted. They are more common about the teeth of young persons and are relatively less common or even rare in older persons. They may be found in the adjacent bone, especially in that of the apical region, and not infrequently in pulp canals of the teeth.

In addition to the epithelium of Hertwig's sheath, that is, the layer adjacent to the developing root, there are other sources of epithelium associated with pathologic processes in this region, namely, the outer epithelial layer of the enamel organ, the inner layer adjacent to the enamel cap, the epithelial extension (cord or lamina) from the mucosa to the dental follicle and the epithelium of the mucous membrane.

On the basis of the foregoing conclusions, the genesis of a dental root cyst may be explained on the assumption that under the stimulation of the infectious process at the apex the epithelial rests in the immediate vicinity increase in size and proliferate into the granulation tissue. At a central position degeneration and liquefaction take place, and in time this cavity has a wall that is continuously lined by this epithelium, except for a defect into which in many cases the apex of the tooth may be inserted. Even in such cases the epithelial lining of the cavity is snugly attached like a collar to the denuded surface of the root. Grawitz (*Die epithelführenden Zysten der Zahnwurzeln*, Greiswald, J. Abel, 1906) had little regard for this theory, and insisted that the epithelial lining of the wall of the cyst was always derived from the mouth by extension along a fistulous tract which at one time had connected the infected region with the mouth cavity. In his opinion, a closed cyst arises from the obliteration of the channel from the mouth after the cavity of the cyst has been lined with epithelium. He believed that only true dentigerous cysts can be derived from the epithelium of the tooth embryo. It is difficult, although not impossible, to find supporters of Grawitz' contention today. James and Counsell (*Brit. Dent. J.* 2:463, 1932) stated that there is no reason to believe that the epithelial rests play any part in the production of pathologic epithelium.

Most writers, however, would agree with the opinion expressed by Siegmund and Weber (*Pathologische Histologie der Mundhöhle*, Leipzig, S. Hirzel, 1926, p. 184), that the epithelium which is found not only in dental cysts but also frequently in granulomas has its origin in the epithelial rests of the Hertwig sheath, which are to be found in the periodontal membrane of every tooth. They said: "In not one case in our abundant material have we been able to demonstrate any connection in the sense of Grawitz between mouth epithelium and that of a granuloma through a fistulous tract."

I have seen two such tissues, but in these it was difficult to decide whether epithelium from the mouth had proliferated into the granuloma, or vice versa.

In one it seemed that the epithelium from the apex of the tooth had grown out toward the mucosa. The fact that cysts of the roots of teeth in the upper jaw are occasionally lined with columnar, ciliated epithelium also suggests that in some instances the lining of the cyst may be formed by the proliferation of epithelium from outside sources instead of from epithelial rests.

Since epithelial rests are found in pulp canals, and because of the peculiar relationship of the cells of inner enamel epithelium to the production of dentine, the close association of these rests and denticles is a matter of no little interest. It can be shown that either all or nearly all true denticles have one or more epithelial rests in close approximation to the border in the same relationship as that of the inner enamel epithelium to the first formed dentine. Calcified nodules in the periodontal membrane may have the same relationship to rests, or the nodule may have the rest as a nucleus into which the lime salts have been precipitated. In the bifurcations of the multirooted teeth, especially of the upper molars, globular deposits of enamel are occasionally found. These are, without exception, associated with larger aggregations of enamel organ epithelium and are usually called enamel pearls. It would seem that their origin is brought about by the activity of that portion of the Hertwig sheath that may have to do with the control of the form and number of the roots. It is possible that at times active ganoblasts are gathered into such a rest, and that in this way the deposition of enamel in a sharply localized spot is brought about. Many of these deposits are so small that they are seen only in histologic preparations.

Odontomas are more likely to develop during the tooth-forming period. They are frequently associated with either missing or retained teeth, and typically they contain all the hard structures of a tooth. Practically the same may be said of dentigerous cysts. Schürmann, Pflüger and Norrenbrock (*Die Histogenese ektomeso-dermalen Mischgeschwülste der Mundhöhle*, Leipzig, Georg Thieme, 1931) described two typical odontomas in the hypophysis. These facts support the belief that the epithelial remnants of the Hertwig sheath have nothing to do with either odontomas or dentigerous cysts, both of which are more nearly akin to supernumerary teeth and are to be considered as arising from a developmental fault in a missing temporary or permanent tooth or from an accessory tooth germ. In hypophyseal tumors with supernumerary teeth rather remote from the mouth, the primitive dental lamina, instead of developing and extending into the tooth-bearing zone, becomes caught and retained in other parts of the head which have an embryologic connection with the oral cavity.

The epithelium of the adamantine tumor and the multilocular cyst resembles that of either the enamel-forming portion of the dental follicle or the dental lamina of the very primitive tooth bud. The adamantinoma frequently contains elements that highly resemble the stellate reticulum of the enamel-forming cap. The cells of both tend to a columnar form and are characteristically different from the cuboidal or nearly round or oval cells of the Hertwig sheath or the epithelial debris. On this account it is generally believed that both arise from either the dental lamina or the tooth bud (*Zahnanlage*). There is little reason, therefore, to consider that Malassez' epithelial debris has anything to do with the genesis of either the adamantinoma or the multilocular cyst. The histogenesis of the adamantinoma is still obscure and is worthy of further study.

As to primary carcinoma of the jaw bones, the situation is even less clear. The carcinoma may arise from any epithelium located in this region, including, of course, the epithelial bodies in the periodontal membrane. Such tumors are extremely rare, although they are no less interesting. The presence of epithelium both in normal periodontal structures and in granulomas is almost constant. On this account alone it does not seem reasonable to lay any great stress on the probable connection between such a source of epithelium and carcinoma. Certainly all cases of carcinoma of the jaw bone merit careful study and serious consideration before sweeping conclusions are drawn.

As the epithelium of the granuloma, the odontoma and the mildly malignant adamantinoma arises from parts of the tooth germ, there is a certain resemblance in the pathologic processes in which they are concerned. On the other hand,

there are distinct differences. The epithelium of the granuloma retains most of the characteristic properties of the parent structure; it appears to proliferate only under the irritation of the infection or as a result of increased metabolism; it undergoes cystic degeneration of a wholly benign character, often with the abundant production of cholesterol, and it never produces enamel, although it may stimulate other forms of calcification. The cysts and granulomas which are associated with it are not neoplastic but are inflammatory reactions. In the odontoma the epithelium forms enamel; it proliferates but does not tend to infiltrate. The growth is benign and encapsulated and may undergo cystic change, with the formation of a dentigerous cyst. Adamantine epithelium proliferates and tends to infiltrate, though it may be sharply limited at times. It tends to recur after excision, never produces enamel and is prone to cystic degeneration, with the formation of a multilocular cyst having the same degree of malignancy as the solid adamantinoma. It would seem to be relatively easy to differentiate between the epithelium of the relatively rare adamantinoma and that of the common granuloma.

Malassez' discovery of the epithelial débris in the periodontal membrane is now apparently universally accepted. His theory as to its origin is also in almost equally good standing. On the other hand, his theories as to its significance in pathologic processes have been confirmed only in part and are thought to be limited to certain processes that are inflammatory and have to do with the genesis of the dental granuloma and cyst. The epithelial débris of Malassez and the so-called glands of Serres are distinct entities.

#### ATYPICAL AMYLOIDOSIS. ELEANOR M. HUMPHREYS.

A man, aged 62, had puzzling symptoms related to the cardiovascular system, the liver and bile passages, and, terminally, the kidneys. These were explained in part by extensive sclerosis of the smaller arteries, associated with thrombosis of a coronary artery, with a myocardial infarct and nephrosclerosis and with marked renal atrophy. The most interesting feature of the autopsy was widespread amyloidosis involving many tissues which are not frequently affected. Amyloidosis of the heart was probably responsible for some of the cardiac symptoms and for the bizarre character of the electrocardiogram. Exceptional features of the case were the unusual amyloid transformation of the testes, the atypical distribution of amyloid in the liver and kidneys and its complete absence from the spleen. Another point of interest was the failure to find a cause for the amyloidosis, unless a seemingly old and healed pleural inflammation or a postoperative subdiaphragmatic abscess which developed two months before death can be implicated. Clinically, there was a marked decrease of the serum protein and of the albumin-globulin ratio, but whether this was related to the amyloidosis is a matter of speculation.

Several similar cases were reviewed.

#### RETOTHEL SARCOMA. PERRY J. MELNICK.

Retothel sarcoma is a term suggested to Roulet by Rössle. He used it in articles published in 1930 and 1932 to replace the term reticulum cell lymphosarcoma. The derivation is from the German *Retothelien* (reticulum). The term is more accurately used, however, as a contraction of the bulky term reticulo-endothelial, because the reticular element should not be separated from the endothelial element of this system.

Reticulum cell lymphosarcoma, or retothel sarcoma, was recognized as a type of lymphosarcoma by Ghon and Roman in 1916. Because the relationship of the lymphoblast to the reticulum cell was not clear, it had been considered as related to lymphoblastic lymphosarcoma. Roulet clearly demarcated it from the latter. The present study includes four cases which came to autopsy and six biopsy specimens of retothel sarcoma at Cook County Hospital. The diffi-

culty of its histologic diagnosis is emphasized, especially in regard to its differentiation from atypical forms of Hodgkin's disease, from mycosis fungoides and from lympho-epithelioma of the pharynx.

## DISCUSSION

VICTOR LEVINE: Retothel sarcomas are not difficult to diagnose. Dr. Melnick will agree that the diagnosis can be made from a simple biopsy.

P. A. DELANEY: I think that terminologists should be commended for their power of invention. I suspected that rethothel might be a contraction of the term reticulo-endothelial, but so far as derivation is concerned, it is poor usage. I much prefer the first recommendation of reticulum cell lymphosarcoma.

## VARIX OF THE PULMONARY VEIN. BENJAMIN H. NEIMAN.

To the three cases of varices of the pulmonary vein already recorded another is added. This occurred in a white woman, aged 57, who complained of cyanosis, clubbing of the fingers and pain in the chest of several years' duration. Roentgen examination revealed a density at the base of the left lung which, on comparison with a roentgenogram taken three years before, showed no change in size or shape. The diagnosis rested between aneurysm of the descending aorta and benign neoplasm of the lung. Fluoroscopically, it was possible to separate this tumor from the heart shadow and show that it had no relation to the aorta. A huge varix of a branch of the second order of the left upper pulmonary lobe, with mural thrombi, was demonstrated post mortem.

The shadow that such a varix casts on the x-ray film must be differentiated from that seen in primary chondroma and solitary echinococcus cyst of the lung. Although the periphery of all three is nodular, that of the varix is smooth, while the echinococcus cyst usually has slight inflammatory infiltrations in the immediately adjacent pulmonary tissue. The periphery of the chondroma is also smooth, but invariably it shows a patchy, irregular distribution of lime salts, due either to simple calcification of the cartilaginous matrix or to true ossification.

If roentgen examination shows a density with a nodular periphery in the lung, and if there are symptoms of cyanosis, dyspnea, cough, clubbing of the fingers and recurrent hemoptysis, without serologic or clinical evidence of infestation with echinococci, varix of the pulmonary vein should be considered.

## Book Reviews

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Comptes rendus de la première conférence internationale de pathologie géographique, Genève, 8-10 Octobre, 1931. Publiés au nom du comité directeur de la Société internationale de pathologie géographique, par M. Askanazy, Président. Paper. Price, 10 francs (Swiss). Pp. 367. Genève: Librairie Kundig, 1933.

This volume records the results of the first concerted international study of a disease. National committees working in twenty-four countries collected information on the occurrence, clinical manifestations, pathologic anatomy, etiology and other features of cirrhosis. This material has been digested, summarized and compared with observations previously recorded. The results are presented under appropriate subdivisions.

Hepatic changes in cirrhosis are summarized by de Josselin de Jong, Utrecht. Materials from the survey and in the recent literature are correlated. The structural features essential to cirrhosis are: (1) increase in connective tissue, (2) degeneration and destruction of liver cells and (3) regeneration of liver cells. Other changes, such as pigmentation, proliferation of bile ducts, infiltrations and vascular changes, are inconstant though often important features. Cirrhosis develops when, through a long-continued diffuse injury, liver cells are destroyed, followed by regeneration and proliferation of uninjured cells and by infiltration and proliferation of reticulum. The essential character of cirrhosis, a chronic inflammatory process, is obscured by attempts at a rigid morphologic classification. "Il n'y a qu'une cirrhose" (there is only one cirrhosis). Morphologic variations result from variations in the character, the intensity and the duration of the injurious agent and in the physiologic reactions of the individual. The etiology includes those agents which, singly or in combination, may produce chronic diffuse inflammation of the liver. Among these are toxic substances and infections. "The importance of alcohol as an etiologic agent has been greatly overrated."

The grouping of cirrheses is regarded not as a classification but as a basis for correlating clinical manifestations with structural changes. Laënnec's cirrhosis as a type is not sharply differentiated as to either etiology or clinical or histologic characteristics. No differentiation is made between Laënnec's cirrhosis and fatty cirrhosis. Pigmentary cirrhosis is regarded not as an entity but as a combination of ordinary cirrhosis with metabolic disturbances of uncertain origin. Cirrhosis may produce the clinical syndrome described by Hanot, but his description of the structural changes is not supported by postmortem evidence. No criteria are found by which to differentiate Banti's disease from Laënnec's cirrhosis. The evidence is insufficient for regarding Banti's syndrome as a disease entity. The occurrence of cirrhosis shows striking geographic variations. In Switzerland cirrhosis is found in more than 10 per cent of autopsies; in adjacent countries the occurrence is less than 3 per cent.

Changes in other organs accompanying cirrhosis are summarized by R. Rössle, Berlin. The indefinite etiology of cirrhosis makes it difficult to distinguish between changes genuinely related and those occurring incidentally. Icterus is a feature in from 20 to 50 per cent of cases. Ascites is found in from 20 to 65 per cent of cases. Splenic involvement, consisting of varying degrees of fibrosis of pulp and the occurrence of perisplenitis with adhesions, is frequent. The spleen is increased in size in about 50 per cent of cases. The frequency with which death results from peritonitis, endocarditis or erysipelas is significant.

The clinical phases of cirrhosis are summarized by Noël Fiessinger, Paris. He comments on the geographic and racial variations. Cirrhosis occurs in the white race more frequently than in Negroes, in a ratio of 3:2. Density of population is accompanied by an increased incidence of cirrhosis. Fiessinger believes that alcohol

is an important etiologic factor in Europe, and that in tropical countries parasitic diseases are more important. Syphilis plays an indefinite, minor etiologic rôle. Discussing clinical types, he states that the syndrome of Hanot exists, but without structural alterations of distinctive character. Clinical manifestations and their significance, tests for hepatic function, the relation of splenic disturbances to cirrhosis, the progressive character of cirrhosis and the terminal complications are discussed.

Experimental cirrhosis in relation to the etiology of human cirrhosis is summarized in six pages by W. E. Gye, London. The subject matter is not well chosen. This phase of cirrhosis is inadequately covered.

Disturbances of metabolism in cirrhosis are summarized by F. C. Mann and J. L. Bollman, Rochester, Minn. Material for this report was drawn from experimental work. The cirrhotic process may produce no measurable metabolic effects except as it impairs the secretion of bile or inhibits the regeneration of hepatic cells. A small amount of hepatic tissue may function adequately without evidences of hepatic insufficiency. Acute degenerations of liver cells are usually found. Mann and Bollmann believe that these are not specific for cirrhosis and result chiefly from impairment of biliary secretion. Cirrhosis produces no marked basal metabolic variations. An important feature of cirrhosis is a decreased glycogen content of the liver cells. This diminishes the detoxicating function of the liver and its ability to resist toxic agents and other injuries. Animals with greatly reduced hepatic tissue do not survive on a diet high in protein. They react severely to doses of toxic substances which are harmless to normal animals. They succumb to surgical procedures from which normal animals recover. The liver has numerous metabolic functions. All of these will be affected by a serious reduction of hepatic tissue.

A report on the frequency and forms of cirrhosis in Stockholm by F. Henschen and T. Bruce and one on statistical data, classification and etiology by P. Robert, Zurich, complete the survey. A verbatim report is made of all discussions. The conference was trilingual: French, German and English. Each item is recorded in the language used in its presentation. The reports reflect a transition of opinion as to etiology, forms and interpretations. Those interested in the morphologic, physiologic or clinical features of cirrhosis will find valuable material in this book.

The organization of the Association was completed and its administration placed in the hands of a directoral committee in which France, Germany, Holland, Switzerland and the United States are represented. An international conference will be held triennially. The second conference will meet in July, 1934, at Utrecht, de Josselin de Jong, president, presiding; the subject is arteriosclerosis. It is gratifying to note that there is an international association for the coordinated study of the various phases of pathology. Its method of procedure promises programs of unique character and high quality.

**Die normale und pathologische Physiologie der Milz.** By Dozent Dr. Ernst Lauda, Assistent der il. medizinischen Universitätsklinik in Wien. Price, 18 marks. Pp. 277, with 2 illustrations. Berlin: Urban & Schwarzenberg, 1933.

This book presents a good review of the literature on the functions of the spleen in health and in disease. The scope is indicated by the list of topics considered in separate chapters: the spleen as a reservoir for blood, particularly red cells; the formation of blood in the spleen; splenic hemolysis; the protective functions of the spleen against infections; the resistance of the spleen to the growth in it of malignant tumors; the relations of the spleen and the gastro-intestinal tract; the spleen and metabolism; the internal secretion of the spleen. At the end of each chapter are a succinct summary of the contents and a useful bibliography. The final chapter gives a cautiously worded digest of the review.



Lauda's book has solid worth for those who are interested in the spleen. The functional relations of this structure are complex; it shares important functions with the reticulo-endothelial system and perhaps also with other systems, and acts too as a more or less independent functional unit. There is, however, no disease so far as is known that is due to hypofunction or hyperfunction of the spleen. Lauda finds that the claims for hyperfunction in certain hemolytic states and in thrombopenia have not been established. Possibly polycythemia as seen occasionally after splenectomy and in splenic tuberculosis may be due to the loss of splenic function. In the embryo the spleen is one of the seats of formation of red cells and myelocytes, and in postembryonal life it shares in comparatively moderate degree in the production of lymphocytes. In infection the spleen has preventive functions of varying significance; it is an important factor in the elaboration of antibodies; it is reasonable to assume that the great splenic swelling in certain infections is associated with increase in protective function; in infection with *Bartonella* in rats the protective action of the spleen is life-saving, and this appears to be the spleen-specific function that is best established. The spleen is of importance as a depot or reservoir for red cells, notably in the dog. Splenectomized rats succumb to poisoning with carbon monoxide, whereas the normal rat survives. The spleen also appears as an independent unit in its relations to malignant tumors and freedom from metastasis. The appearance of Howell-Jolly bodies in the circulating blood after splenectomy is mentioned as perhaps also an indication of a special splenic function. The book is an important and helpful addition to the literature on the spleen.

**Bacterial Infection. With Special Reference to Dental Practice.** By J. L. T. Appleton, Jr., B.S., D.D.S., Professor of Microbiology and Bacteriopathology, the Thomas W. Evans Museum and Dental Institute, School of Dentistry, University of Pennsylvania. Second edition, enlarged and revised. Cloth. Price, \$7. Pp. 654, with 122 engravings and 4 colored plates. Philadelphia: Lea & Febiger, 1933.

The purpose of this book is "to aid the reader to form a comprehensive concept of infection" and "to point out wherever a knowledge of infection will help the dentist in understanding or solving his problems." The author takes it as granted that if there is to be close and effective cooperation between dentist and physician, the dentist must have a knowledge "equivalent to that possessed by the physician, of what infection means." The book is in three parts. The first part deals with the morphology, physiology and ecology of bacteria. The second part treats of infection as a whole, its nature, modes of action, types, dissemination; the protection and responses of the host; immunity; prevention, including oral hygiene. The third part is devoted to a comprehensive presentation of the infections of the oral cavity. Most of the illustrations are taken from other books. There are abundant references to the literature. The style is clear, fluent, readable. The author shows a remarkable familiarity with the current literature, especially the English and German. The use of italics in cumbersome scientific or proper names of common bacteria, when not required for purposes of classification or identification, is not desirable. Why say *Corynebacterium diphtheriae*, *Eberthella typhi*, *Escherichia coli*, *Myobacterium tuberculosis* and *Neisseria gonorrhoeae* when diphtheria bacillus, typhoid bacillus, colon bacillus, tubercle bacillus and gonococcus will answer the purpose fully? Generally speaking, the teachings of the book are sound. It contains an enormous amount of detailed, minute information about infection and allied topics. As a textbook for students it is too elaborate. To require students of dentistry to master its contents is neither humane nor good pedagogics. As a book of reference for dentists and physicians it merits recommendation. In future editions the effort should center on condensation, simplification and sound generalizations.

**The Great Doctors. A Biographical History of Medicine.** By Dr. Henry E. Sigerist, Professor of the History of Medicine, The Johns Hopkins University. Translated by Eden and Cedar Paul. Price, \$4. Pp. 436, with 68 illustrations. New York: W. W. Norton & Company, Inc., 1933.

This book is a translation from the second German edition. The first edition was published in 1931 and was well received. As indicated in the subtitle, a biographic history of medicine is given, made up of short sketches, never longer than ten or eleven pages, of fifty great figures of vital importance in the development of medicine. The first chapter presents Imhotep and Aesculapius, the second Hippocrates and the late Osler. The plan of the book excludes more than passing allusion to names of living persons; there is no aim at encyclopedic completeness; attention has been restricted to a small number of creative masters of representative significance in each epoch. It does not seem advisable to consider each of these sketches in detail. They are written by a ready and competent pen. The sketches of the following are mentioned as of special interest to pathologists in the strict sense: Morgagni, Rokitansky, Bernard, Virchow, Pasteur and Koch. By giving the subject of each sketch the proper relative historical setting, a broad conception is conveyed of the growth of medical knowledge. The book is dedicated "To the Unknown Doctor who in unselfish and inconspicuous activities fulfills the teachings of the Great Doctors." The illustrations, sixty-eight in all, add greatly to the interest of the book. Physicians and medical students will find this a book of exceptional interest and stimulus.

## Books Received

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THE ERADICATION OF BOVINE TUBERCULOSIS. L. Jordan. Medical Research Council, Special Report Series, No. 184. Price, 2s., net. Pp. 104. London: His Majesty's Stationery Office, 1933.

METABOLIC DISEASES AND THEIR TREATMENT. Dr. Erich Grafe, Professor of Medicine of the Clinic of Medicine and Neurology, University of Würzburg, Germany. Translated by Margaret Galt Boise under the supervision of Eugene F. DuBois, M.D., Medical Director, Russell Sage Institute of Pathology; Professor of Medicine, Cornell University Medical College, New York, and Henry B. Richardson, M.D., Associate Professor of Medicine, Cornell University Medical College, New York. Price, cloth, \$6.50, net. Pp. 551, with 37 illustrations. Philadelphia: Lea & Febiger, 1933.

HISTOLOGY. S. Ramón y Cajal, M.D. (Madrid), F.R.S. (London), LL.D. (Clarke), Director, Royal Cajal Institute for Medical Research; Emeritus Professor of Pathology, University of Madrid Faculty of Medicine; Nobel Premiate in Medicine; Life Senator of Spain. Revised by J. F. Tello-Munoz, M.D. (Madrid), Professor of Pathology, University of Madrid Faculty of Medicine. Authorized translation from the tenth Spanish edition by M. Fernán-Nunez, M.D. (Madrid), Professor of Pathology, Marquette University Medical School. Price, \$8. Pp. 738, with 535 illustrations. Baltimore: William Wood & Company, 1933.

MEDICAL USES OF RADIUM: SUMMARY OF REPORTS FROM RESEARCH CENTRES FOR 1932. Medical Research Council, Special Report Series, No. 186. Price, 1s., net. Pp. 36. London: His Majesty's Stationery Office, 1933.

DIET AND HEALTH. Milton T. Hanke. Pp. 236, with illustrations. Chicago: University of Chicago Press, 1933.

STUDIES FROM THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF PENNSYLVANIA. Volume IV. Edited by E. B. Krumbhaar, Professor of Pathology. Philadelphia: University of Pennsylvania, 1932-1933.

THE CHEMISTRY OF FLESH FOODS AND THEIR LOSSES ON COOKING. R. A. McCance and H. L. Shipp. Medical Research Council, Special Report Series, No. 187. Price, 2s. 6d., net. Pp. 146. London: His Majesty's Stationery Office, 1933.

NUMBER 4 CANADIAN HOSPITAL: THE LETTERS OF PROFESSOR J. J. MACKENZIE FROM THE SALONIKA FRONT. With a memoir by his wife, Kathleen Cuffe Mackenzie. Price, \$2.50. Pp. 247, with 13 illustrations. Toronto: Macmillan Company of Canada, 1933.

## MELANIN

### I. ITS MOBILIZATION AND EXCRETION IN NORMAL AND IN PATHOLOGIC CONDITIONS

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AND

GUSTAVUS H. KLINCK, Jr., M.D.

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The numerous problems concerning normal and pathologic endogenous pigmentation offer difficulties in their solution derived from the fact that the absolute identification of a pigment unaltered by extravital chemical reactions is rarely possible. The gradation of colors through the various shades of yellow and brown to black includes substances which often have a certain basic molecular similarity but which, nevertheless, may be the result of fundamentally different cellular activities. Undoubtedly too much importance has been given to the presence of stainable iron or lipoid in differentiating microscopically one colored body from another. Progress in this difficult field of study will necessarily be as slow as improvement in laboratory methods, especially in microchemical technic.

The distribution of hemosiderin, it is assumed, can be determined best by the demonstration in granules of iron through the use of the prussian blue test and its modifications and by the reaction of iron with ammonium sulphide. We shall allow, for the sake of argument, that iron in this form is indicative of hemosiderin. When, however, the iron is identified only after the maceration or digestion of tissues, and then usually in minute quantities and by highly refined chemical methods, it cannot be accepted that the pigment is necessarily hemosiderin.

One of the pigments which occurs in unicellular organisms as well as in Mammalia is called melanin. Its normal distribution in man, according to Bloch,<sup>1</sup> is confined to the skin, the pigment layer of the retina, the ciliary body and the choroid, except for its occurrence in certain parts of the central nervous system. Melanin is difficult to isolate since it does not crystallize and is soluble with difficulty in fluids

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These studies have been aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Bloch, B.: *Am. J. M. Sc.* **177**:609, 1929.

which offer the least possibility of altering its complex molecule. From this fact alone it seems unwise to limit its normal presence to the skin and the eye, since other morphologically similar pigments are found elsewhere in the body which, when deprived of loosely held sulphur or iron, for instance, may prove to be members of this group of endogenous pigments.

Since melanin is formed normally within the body by the melanoblasts and is transported by the melanophores, a certain amount of the pigment must constantly be disposed of in some way following the obsolescence or disintegration of the cells containing it. What is the fate of this pigment? Some of it undoubtedly escapes into the intestinal canal. Melanosis of the colon is encountered in chronically constipated persons, the pigment being found in phagocytes in the mucosa. The mucosa of the appendix frequently contains melanin. Does the kidney play a part in the excretion? In a note on melanins, Hawk<sup>2</sup> stated:

These pigments never occur normally in the urine, but are present under certain pathological conditions. . . . In many instances, without doubt, urines rich in indican have been wrongly taken as diagnostic proof of melanuria. . . . The pigment melanin is sometimes mistaken for indigo and melanogen for indican. . . . In rare cases melanin is found in urinary sediment in the form of fine amorphous granules.

The kidney of the adult often contains a yellowish-brown pigment in the form of coarse granules in the epithelium of the collecting tubules and Henle's loops and, less frequently, in the convoluted tubules. This pigment gives a negative reaction for iron but appears to contain a lipid from its reaction with fat stains and osmic acid. Since the Negro produces normally the greatest amount of melanin found in man, it should follow that he would show the greatest normal excretion of the pigment.

This study is an outgrowth of a more comprehensive survey of the significance of melanin in normal and in pathologic cells. A fundamental difficulty lies, of course, in the inadequacy of the methods of identifying melanin. Its argyrophilia, however, offers an approach which Foot<sup>3</sup> has utilized in his work with benign and malignant melanotic tumors. Using his method, with a slight modification, we have studied the distribution of melanin in the normal kidneys of adult Negroes, in patients with Addison's disease, in Negroes with malignant melanoma with generalized metastases, in blonde and brunette white persons and in more than four hundred white mice, each of which had a melanoma derived from one originally discovered by Harding and Passey<sup>4</sup> at Guy's Hospital in 1915 and obtained by us from Sugiura of the Memorial

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2. Hawk, P. B., and Bergeim, O.: *Practical Physiological Chemistry*, ed. 10, Philadelphia, P. Blakiston's Son Co., 1931, p. 776.

3. Foot, N. C.: *Am. J. Path.* **7**:619, 1931.

4. Harding, H. E., and Passey, R. D.: *J. Path. & Bact.* **33**:417, 1930.

Hospital, New York. The technic of Foot is only one of many technics based on the use of silver nitrate, but it is very satisfactory for the demonstration of melanin as well as of reticulum.

Many kinds of cells or their inclusions or processes may be impregnated by silver salts under certain conditions; hence it is advisable always to state by what method their argyrophilia is demonstrated. The term argyrophilia is a bit vague, as Nageotte of Paris stated to Laidlaw,<sup>5</sup> but what it should mean is the relative degree of affinity for silver salts displayed by one type of substance as compared with another under similar conditions. We have had much experience with Foot's



Fig. 1.—A white mouse with a large intraperitoneal Harding-Passey melanoma resulting from the intraperitoneal inoculation of an emulsion of mouse melanoma two hundred and twenty-four days previously. There are a few small metastases in the liver and the lungs. The kidneys showed marked melanosis.

silver methods, and in order that a comparison of results might be based on a uniform technic, his bromuration procedure has been used, largely to the exclusion of other variations.

The isolation of melanin in a pure state is apparently impossible at present because of its relative insolubility *in vitro* without the aid of a strong alkali or acid. It must be soluble in certain body fluids or it possibly exists as a colloidal suspension, because either it or its precursor, melanogen, is present in the urine in many cases of melanoma, and,

5. Laidlaw, G. F.: Personal communication to the authors.

while it is phagocytosed by various cells, it has never, to our knowledge, been detected in particulate form in the cells of the circulating blood. While melanin has apparently not been seen in circulating leukocytes, Davis <sup>6</sup> has noted its ingestion by white blood cells and also by cells of the renal tubules and entodermal cells of the intestine in tissue cultures. Since melanin is a constant component of many cells of the body, it follows that the pigment must be disposed of in some way when melanophores die; the ordinary catabolic processes probably destroy but a small amount of it. Odiorne <sup>7</sup> has shown that a certain amount of melanin escapes from the body of the killifish (*Fundulus heteroclitus*) in the desquamation of epithelium from the pigmented spots. This method undoubtedly accounts for the loss of much melanin from the integument in all pigmented species.

The accurate identification of melanin either in vivo or in vitro is not possible for the reasons already given. Yet there is much literature on melanin, and dogmatic statements have been made and probably will continue to be made concerning this substance, at least until a microchemical test has been devised which is above criticism. This will be possible only after the chemical composition of melanin is definitely known. As things stand, much of the proof that a given pigment is melanin is circumstantial. First, it may be found in cells which give a positive dioxyphenylalanine reaction, that is, cells capable of oxidizing 1-3-4 dioxyphenylalanine to a pigment indistinguishable from melanin. These cells are regarded as melanoblasts in contradistinction to cells which can only phagocytose melanin but cannot manufacture it. This conception hinges on the absolute specificity of Bloch's dopa reaction as indicating melanoblasts only. There are still several weaknesses to be remedied in the application of this reaction before it can be approved in this degree, but the outlook is good for final acceptance.

Second, melanin may be seen as a dark brown granular pigment in the tumor arising from the cells of a pigmented nevus, whether or not the dioxyphenylalanine reaction is studied; this pigment is negative for stainable iron.

Third, it may be found in fibroblasts, monocytes and reticulo-endothelial cells adjoining an area of pathologic pigmentation, for instance, a melanoma, or in the skin in a case of excessive melanosis, such as Addison's disease.

The use of frozen sections has seen wide application in recent years in the rapid diagnosis of tissue, in the study of the distribution of fats and lipoids and in the demonstration of nerve fibrils and reticulum. It has long been known that melanin is capable of reducing silver salts,

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6. Davis, D. T.: Bull. Johns Hopkins Hosp. **32**:240, 1921.

7. Odiorne, J. M.: Proc. Nat. Acad. Sc. **19**:329, 1933.

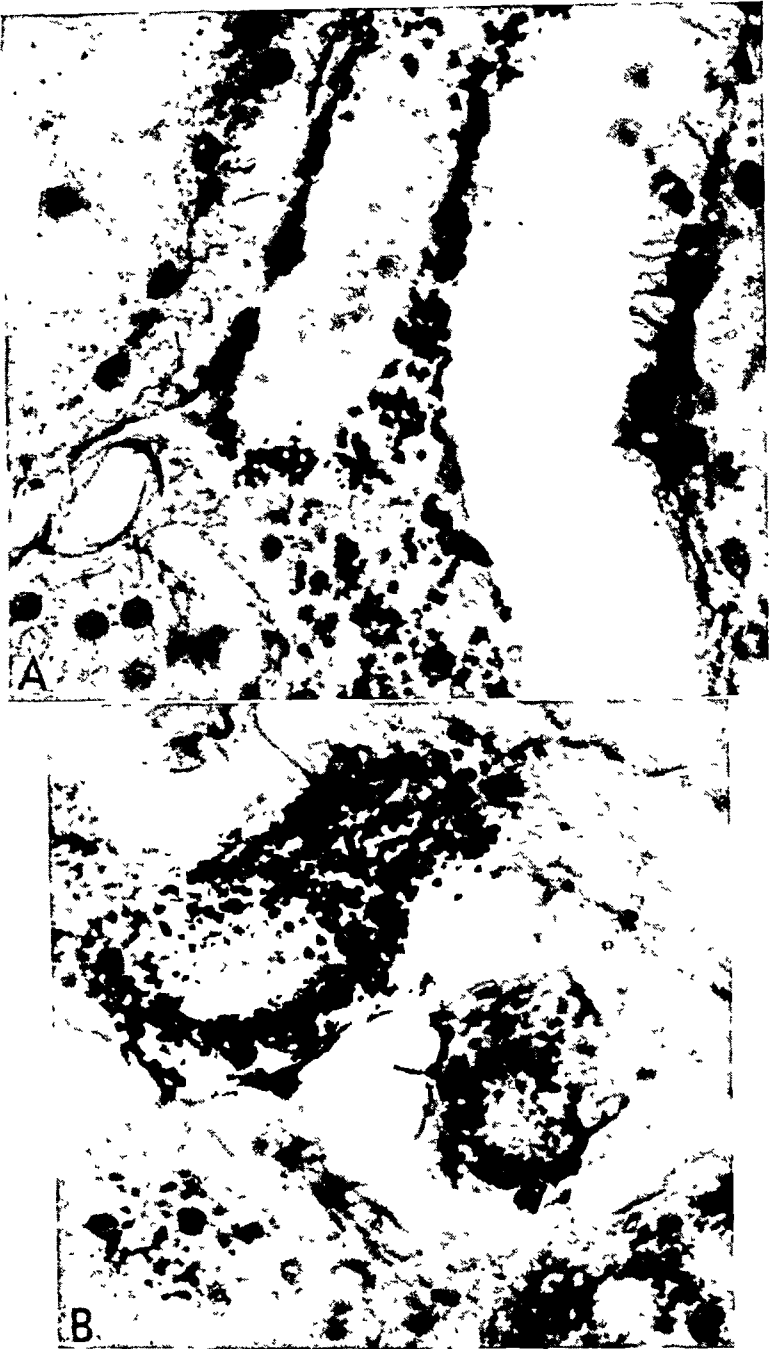


Fig. 2.—*A*, renal tubules in the kidney in Addison's disease. Melanin granules are seen in the epithelial cells and in the lumen. *B*, renal tubules in a mouse with a subcutaneous melanoma one hundred and seventy-nine days old. The melanin granules are somewhat scattered through the cytoplasm, but tend to be grouped in the midzone. Foot's silver-bromuration method.



and with the impetus given to the study of the nervous origin of pigmented nevi by Masson a renewed interest in the argyrophilia of melanin has been manifest in the most recent studies of the melanomas. However, until the identity of all the granules blackened by silver which are found in cells fixed in a solution of formaldehyde is determined, there will continue to be skepticism in regard to the conclusions drawn from the use of this method. At present it is necessary to consider all the circumstances surrounding the presence of the pigment in question, and in our study we have tried so to do. Our conclusions are based on the consideration mentioned, and it is hoped they do not seem too dogmatic when viewed in that light.

In cases of pathologic increase of melanin in the adult human being with Addison's disease or melanomatosis, the kidneys contained much yellowish-brown or deeper brown pigment in the epithelium of Henle's loops and in many collecting tubules, as well as in the reticulo-endothelial cells of the liver, spleen and lymph nodes. These pigmented granules were present in fresh unstained frozen sections, in preparations fixed in a 10 per cent solution of formaldehyde and in Zenker's fluid embedded in paraffin and stained with hematoxylin and eosin or with eosin and methylthionine chloride, U. S. P. (methylene blue). The alcohol and chloroform removed any carotene present, since it is soluble in these fluids. Other lipoids and fats were similarly extracted, leaving only a pigmented residue which was still capable of reducing silver nitrate and of being bleached by hydrogen dioxide, and the identity of which we have sought to establish.

In the renal tubules the pigment is usually found in the peripheral portion of the epithelial cell in small dustlike granules or larger agminate masses; the color varies from dirty yellow to brown, depending on the amount of pigment present. This pigment is usually called "lipochrome" but has not been regarded as melanin, so far as we are aware. Yet it occurs in large amounts in many patients with pathologic melanosis, in elderly brunettes and in constipated Negroes. It differs in no essential respect from the pigment in the mucosa of the colon in melanosis coli. It is strongly silver positive. The pigment is found in the lumen of the tubules, either in desquamated epithelium or free, having possibly been discharged from the cells or precipitated from its solution in the fluid in the tubules. It is often found in renal casts in the same manner as hemosiderin is found in hemochromatosis or pernicious anemia. In one of our cases of Addison's disease, proved by necropsy, strongly silver-positive, yellowish-brown granules were demonstrated before death in renal casts from the urinary sediment (fig. 3 *A*), and the kidneys were found to contain much pigment in the locations already noted. Similar deposits of the pigment were encountered in the kidneys of several patients with malignant melanomatosis.

In our study of the organs of white mice harboring the transplantable Harding-Passey melanoma, melanosis of the intestines was never seen. Melanosis coli is found in man only in extreme constipation. In these mice with tumors of large size, i. e., 1.5 cm. and larger, renal

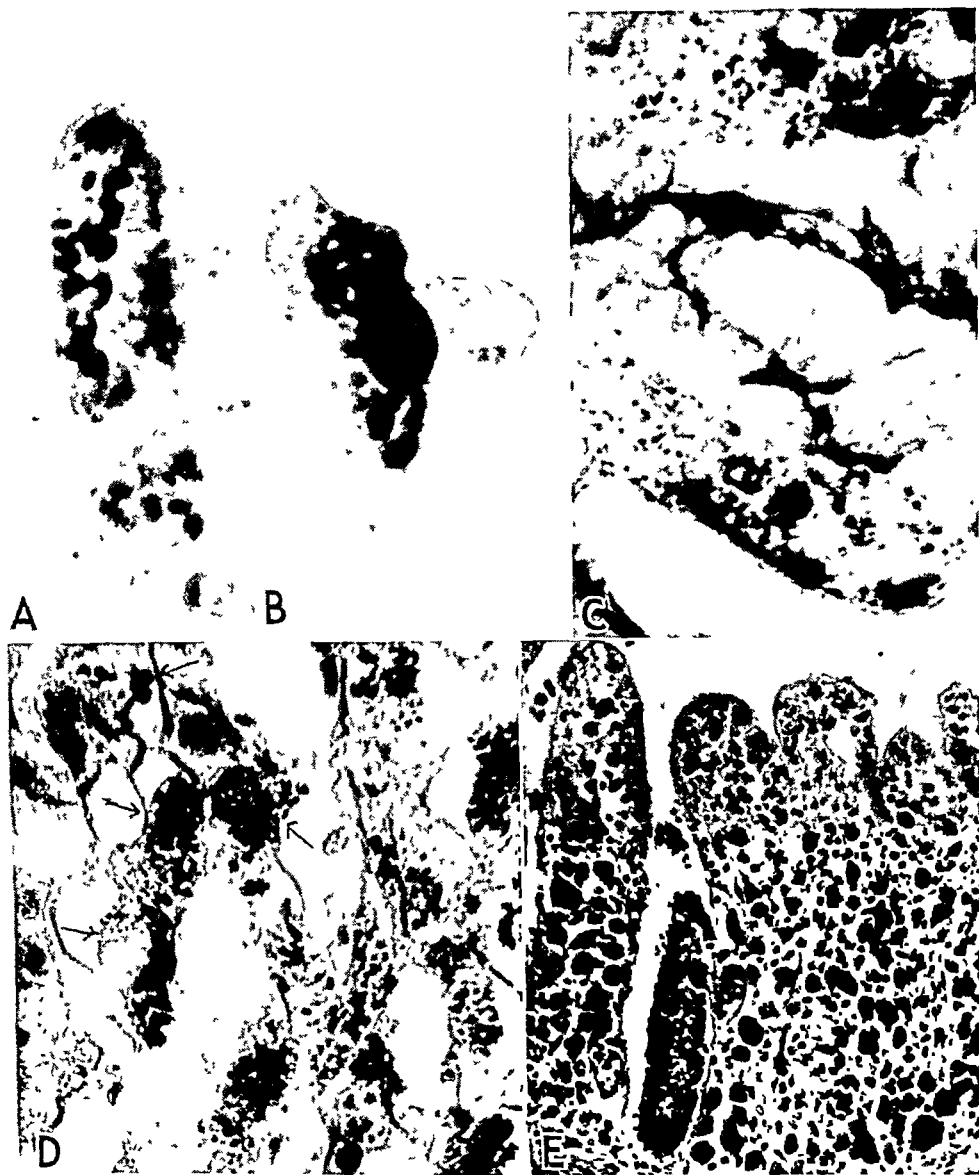


Fig. 3—*A*, a pigmented renal cast in the urinary sediment of a patient with Addison's disease. *B*, a pigmented epithelial cell in the urinary sediment of a patient suffering from generalized melanosis with diffuse myelomatosis. *C*, renal tubules in a Negress with melanomatosis. Melanin granules are seen in the epithelium and in the tubular casts. *D*, section of the suprarenal gland of an elderly white man. Melanin granules are abundant in the cells of the medulla on the right. The arrows indicate the cells of the zona reticularis containing melanin. *E*, melanosis coli. The pigment is contained in phagocytes in the mucosa.

melanosis was encountered in a high percentage. The animals were fed the same diet as the controls, which never showed any pigmentation of the kidneys. This renal pigment is not soluble in lipid solvents, and its ability to reduce silver is lessened slightly when it has been previously immersed in chloroform and alcohol in the process of dehydration for embedding in paraffin. Dyson<sup>8</sup> noted that a pigment normally of lighter color resulted from employing the solvents used for preparing tissues for paraffin, and he asked if there were two different forms of melanin. Melanin, he stated, is included in the protein part of an excretion of the cell nucleus, a bluish granule of lipid and protein being the precursor of the pigment. The chemistry of melanin will not be discussed in extenso in this paper, but because of its reaction with fat stains and lipid solvents we believe that the melanin molecule often links itself loosely with a lipid complex.

Wells<sup>9</sup> stated that melanuria is not ordinarily observed unless there is extensive development of melanotic tumors, and that it is seen in only about 20 per cent of the cases of melanosarcoma, chiefly when the liver is extensively involved. Formerly the excretion of true melanin or melanogen was supposed to be diagnostic of the presence of melanosarcoma, but melanuria has been observed in persons without tumors of this type and with no other evident source of the pigment. In several cases there was intestinal obstruction, from which the precursors of the pigment may have resulted through the disintegration of protein.

Schreyer<sup>10</sup> noted in the renal epithelium a pigment resembling the lipofuscins, which increases with age and which is not related to the urinary pigments. Brahm and Schmidtman,<sup>11</sup> on the other hand, expressed the belief that lipofuscin is physically and chemically similar to melanin and that it is probably absorbed or dissolved by lipins.

Risak and Asperger,<sup>12</sup> in studying the appearance of melanin reactions in human urine, found that after intense solar irradiation a positive Thormählen reaction developed in the urine of a normally pigmented person, a phenomenon not previously observed in irradiated and tanned persons. In persons poor in pigment this reaction was not observed.

The kidney of the adult Negro frequently contains in Henle's loops and in the collecting tubules granular pigment, which, so far as we can determine, is melanin. There is an increase in renal pigment in the constipated Negro. In white adults, especially in brunettes, who have

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8. Dyson, W.: *J. Path. & Bact.* **15**:298, 1910.

9. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1926, p. 530.

10. Schreyer, H.: *Frankfurt. Ztschr. f. Path.* **15**:333, 1914.

11. Brahm, B., and Schmidtman, M.: *Virchows Arch. f. path. Anat.* **239**:488, 1922.

12. Risak, E., and Asperger, H.: *Klin. Wchnschr.* **11**:154, 1932.

died of severe wasting disease, i. e., diseases accompanied by widespread destruction or atrophy and desquamation of cells (including, of course, the many tissues which either make or contain melanotic pigment), the kidneys usually show melanosis. In melanosis coli the pigment in phagocytes in the colonic mucosa seems to be true melanin and occurs only in very constipated people. As has been stated before, the kidneys of mice with subcutaneous or intraperitoneal melanomas contain much melanin. The intestinal mucosa is free from melanin, but if one could produce chronic constipation in mice with this condition melanosis coli might possibly ensue. Recently Bockus, Willard and Bank<sup>13</sup> suggested that melanosis coli is the result of the use of anthracene laxatives, such as cascara sagrada, but if this were true melanosis coli should be more frequent in view of the wide use of these laxatives. However, little is known about the exact mechanism of melanin formation, and in the intestines such synthesis may be possible, given the proper materials and conditions.

The pigment present in the zona reticularis of the human suprarenal gland has been known by various names; usually it has been dismissed as lipochrome. However, since it is still present after dehydration in alcohol and chloroform it must be something else, as carotene and xanthophyll, the principal lipochromes, are soluble in fat solvents. The pigment which normally gives a yellow color to the entire cortex of the suprarenal gland in young people probably belongs in the category of lipochromes, but the pigment in the zona reticularis of the suprarenal gland in old people, which gives a brown to black color to this layer, is not soluble in lipid solvents and stains an intense black with silver nitrate. It behaves, then, more like melanin than like any other pigment. In our experience, the cells of the zona reticularis have always given a negative dioxypheylalanine reaction. Laidlaw<sup>14</sup> has confirmed this finding. If the pigment is melanin, where does it come from? The epithelial cells of the suprarenal gland are definitely not melanoblasts. Melanin is most likely to be found in the vicinity of melanin-forming cells. The cells of the suprarenal medulla are of sympathetic origin: in other words, they arise in the neuro-ectoderm where all melanoblasts should come from, if the neurogenic origin of melanomas is correct. We have studied the suprarenal gland in many patients of various ages and have found that the granular pigment of the cells of the medulla is not soluble in fat solvents and is very silver positive. Connor regards this pigment as melanin and so do we. In figure 3 *D*, depicting a frozen section of the suprarenal gland of an old person who died of pneumonia, which was treated by Foot's silver-bromuration method, there is present

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13. Bockus, H. L.; Willard, J. H., and Bank, J.: *J. A. M. A.* **101**:1, 1933.

14. Laidlaw, G. F.: Personal communication to the authors.

an abundance of melanin granules in the cells of the medulla. Granules of similar appearance are present in the contiguous cells of the zona reticularis, the pigment becoming less abundant the farther the cells are from the medulla. With higher powers of the microscope there even appears to be an extrusion of melanin granules by the medullary cells. The granules seem to be taken up by the cortical epithelium of the zona reticularis. These epithelial cells of the suprarenal cortex, then, act like melanophores and hence can be added to the group of cells which can carry melanin but cannot manufacture it. The list includes macrophages, endothelial cells and fibroblasts. This explanation of the origin of the melanin in the suprarenal gland harmonizes with the Soldan-Masson theory of the origin of melanotic tumors and makes it seem logical to believe that melanin, wherever it is found, is always of neuroectodermal origin.

#### SUMMARY

The normal distribution of melanin is confined to the skin, the pigment layer of the retina, the ciliary body, the choroid, certain parts of the central nervous system and the medulla and zona reticularis of the suprarenal gland.

While melanin is soluble *in vitro* only in strong alkali or acid, it must be soluble or in colloidal suspension in the body fluids, because it or its precursor, melanogen, colors the urine in many cases of melanoma. The pigment has probably rarely, if ever, been detected in particulate form in the leukocytes of the blood.

The mobilization of melanin from areas of normally or pathologically pigmented cells and the paths of its excretion from the body have been studied in aged blondes and brunettes, in adult Negroes, in patients with Addison's disease, in those with melanoma and in more than four hundred white mice each of which had the transplantable Harding-Passey mouse melanoma.

In Addison's disease and melanomatosis the kidneys contained much melanin in Henle's loops and in the collecting tubules, as did the reticulo-endothelial cells of the liver, spleen and lymph nodes. Melanin granules were often present in renal casts, and one diagnosis of Addison's disease was made *ante mortem* partly on the basis of such a finding. The kidneys of old people, especially brunettes, and of constipated adult Negroes, who died of severe wasting disease showed similar findings. In melanosis coli the pigment in the phagocytes in the colonic mucosa seems to be true melanin and occurs only in very constipated people. The paths of excretion of melanin appear to be, then, from the skin by desquamation, through the intestinal tract and through the kidneys. The ingestion of melanin in foods and the synthesis of melanin in the intestines may account for much of the pigment in melanosis coli.

The pigment of the zona reticularis of the suprarenal gland appears to be melanin, and it is suggested that the pigment is absorbed from the neurogenic cells of the contiguous suprarenal medulla, thus adding the epithelial cells of the suprarenal cortex to the list of possible melanophores. This explanation of the origin of melanin in the suprarenal gland is in harmony with the Soldan-Masson theory of the origin of melanotic tumors and suggests that melanin, wherever it is encountered, is always of neuro-ectodermal origin.

# EXPERIMENTAL EDEMA

## FURTHER EXPERIMENTS ON THE TYPE OF EDEMA PRODUCED BY A DIET LOW IN PROTEIN

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DALLAS, TEXAS

Dr. Egloff and I have described<sup>1</sup> a method of producing hypoproteinemia and edema in a dog by feeding a diet with a low protein content. We have repeated this experiment on four adult dogs, and were able to produce hypoproteinemia of the same degree found in the first dog, but only two of the four animals became edematous when salt and water were forced by gavage. We again found an excess of fat in the renal tubules of the animals. The amount of fat in the animals that had edema and those that had hypoproteinemia without edema was quite similar, which doubtless is proof that the edema itself has little to do with the fatty deposits.

There has been a suggestion that these lesions are accidental findings (Leiter<sup>2</sup>). We have added control studies, lacking in the first paper, which make more convincing the evidence that the fat changes are actually a result of the experiment and not changes found in most dogs used in the laboratory. In the first place, we examined sections of the kidney from eighteen dogs used in various experiments in the laboratory which we were sure did not influence the kidneys. These sections uniformly showed little or no fat in the renal tubules. Also we fed two dogs a high fat but normal protein diet, and these dogs showed very little fat in the renal tubules.

We have been unable to prove that the fatty changes in the renal tubules were the result of protein starvation and low plasma proteins per se, for we could not show that the anemia which invariably appears in these experiments was not a potent factor in the deposition of this fat. However, Dr. George H. Whipple of Rochester, N. Y., sent us sections stained for fat from four dogs which had been kept anemic for more than a year, and only one of these showed an excess of fat in the convoluted tubules. He did not study the plasma proteins, so it is possible that this one animal had hypoproteinemia. Neither Dr. Whipple nor we are willing to admit this as positive evidence. Further experiments are in progress to control this factor.

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1. Shelburne, S. A., and Egloff, W. C.: Arch. Int. Med. **48**:51, 1931.

2. Leiter, Louis: Arch. Int. Med. **48**:1, 1931.

Therefore, we are still unable to convince ourselves that there is a definite relation between the fatty changes and the similar deposits in the convoluted tubules of patients with the nephrotic syndrome. As contrary evidence, we have been able to show that the renal fat in all of our dogs was not doubly refractile. This is of importance, as Lubarsch<sup>3</sup> has shown that this type of fat is characteristic of the nephrotic types of renal disease. Therefore, we report this as an interesting finding that may in the future throw some light on the complex subject of fat metabolism.

## METHODS AND FURTHER COMMENTS

Four adult female dogs, weighing from 15 to 18 Kg., were selected for feedings low in protein. They were fed a normal Cargill synthetic diet and then placed on a diet low in protein. We tried Cargill's diet

TABLE 1.—*Diet A*

Constituents	Grams	Protein, Gm.	Fat, Gm.	Carbohydrate, Gm.
Lactose.....	75	0	0	75.0
Butter.....	50	0.5	42.0	
Cod liver oil.....	30	...	30.0	
Turnip.....	100	1.3	0.2	8.1
Potato.....	100	2.2	0.2	18.4
Bone ash.....	10	0	0	0
Salt mixture.....	2	0	0	0
Total.....	367	4.0	72.4	101.5

Yeast concentrate, 20 Gm. added each week

Total caloric value, 1,078 (from 60 to 70 calories per kilogram for one dog)

low in protein (synthetic), but after a short time the dogs refused this food, so we had to return to a vegetable diet similar to that used in our first experiments, but with the addition of a yeast concentrate, bone ash and a salt mixture (Cargill).

Two other dogs, 5 and 6, were placed on a diet with the same fat content, but with an adequate amount of protein. These animals were considered as proper controls on the factor of the increased fat in the diet affecting the amount of lipoid in the renal tubules.

We were able to produce edema in dogs 1 and 3 after the plasma protein fell below 4 Gm. per hundred cubic centimeters, with the albumin below 2 Gm. It is interesting to note that although very low plasma protein levels were reached in dogs 2 and 4, there was never a time when the total plasma protein was below 4 Gm. and the plasma albumin was below 2 Gm. We were unable to precipitate edema in these

3. Lubarsch, O., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1925, vol. 6, p. 525.



animals in spite of forcing large amounts of salt and water. In summary of our total experience with this type of edema in dogs, we may say that these figures probably represent critical levels of plasma protein for the formation of edema. Of course, this is much lower than the critical levels in man.

We again noticed marked anemia in these animals after prolonged low protein feeding. We attempted to run a control on the part played by this factor in causing the fatty changes in the kidneys by removing

TABLE 2.—*Results in the Case of Dog 1*

Date	Weight, Kg.	Hematocrit, per Cent	Total Plasma Protein, Gm.	Plasma Albumin, Gm.	Plasma Globulin, Gm.	Fatty Acids, Gm.	Cholesterol, Gm.	Serum Phosphorus, Mg.	Blood Urea Nitrogen, Mg.	Diet*	Comment
12/22/30	18.4	....	...	...	...	....	....	...	..	N	
1/ 1/31	19.0	51.0	7.3	5.1	2.2	0.390	0.140	2.3	16	LP	
1/23/31	17.2	42.0	5.5	3.5	2.0	....	....	...	..	LP	
2/ 2/31	15.8	....	...	...	...	....	....	...	..	A	
3/ 2/31	14.4	43.5	5.1	2.7	2.4	0.340	0.187	...	..	A	
3/27/31	13.2	36.0	4.6	2.1	2.5	0.410	0.215	...	..	A	
4/25/31	11.2	36.0	4.1	2.8	1.3	0.350	0.130	...	..	A	
5/20/31	11.2	36.0	3.7	2.3	1.4	....	....	...	4	A	
5/22/31	11.2	....	...	...	...	....	....	...	..	A	1,000 cc. of 1 per cent solution of sodium chloride for 2 days
5/26/31	12.9	33.0	3.6	2.0	1.6	....	....	2.3	..	A	Edema
5/27/31	12.9	....	...	...	...	....	....	...	..	M	Left nephrectomy; edema; transfusion of 450 cc. of blood
5/29/31	13.1	23.0	3.6	2.0	1.6	....	0.217	...	10	M	Edema
6/ 3/31	11.5	33.0	5.8	2.8	3.0	....	0.240	...	8	M	No edema
6/13/31	13.4	32.0	6.1	3.2	2.9	....	....	...	..	M	250 cc. of whole blood withdrawn
7/ 3/31	12.8	31.0	4.2	...	...	0.682	0.172	...	18	M	250 cc. of whole blood withdrawn
7/15/31	12.8	24.0	3.7	...	...	0.607	0.163	...	14	M	340 cc. of whole blood withdrawn
7/18/31	12.8	24.0	3.8	...	...	....	....	...	..	M	Autopsy

\* In this and the succeeding tables, under diet, N indicates the normal Cargill diet (synthetic); LP, the low protein Cargill diet (synthetic); A, the low protein diet (vegetable), and M, the milk (1,000 cc. daily) and meat (about 200 Gm. daily) diet.

one kidney after the plasma proteins had fallen to the edema level, allowing the protein levels to rise by feeding meat and milk, but maintaining severe anemia by bleeding, and then comparing the amount of fat in the two kidneys. Unfortunately the bleeding, or a failure to eat all of the food, resulted in the maintenance of the low plasma protein levels, and so this approach to the problem failed (tables 2 and 3).

All the kidney tissues were fixed in both formaldehyde and Zenker's fluid. Sections were stained with hematoxylin and eosin and for fat with Nile blue sulphate and Sudan III. An unstained section of formaldehyde-fixed tissue was examined in water with polarized light and crossed prisms, both before and after warming.

The gross appearance of the kidneys was not remarkable. However, the renal tubules of the dogs fed a diet low in protein uniformly showed a large excess of isotropic fat when compared with those of the control animals. There was little or no difference in the amount of fat found in the kidneys removed at operation and those removed later at autopsy.

It may also be pointed out that we found no significant changes in the blood cholesterol or fatty acids during the prolonged periods of observation.

#### PROTOCOLS

Dog 1.—This dog was an adult, smooth haired, female which weighed 18.4 Kg. She was fed a normal synthetic diet (Cargill) from Dec. 22, 1930, until Jan. 1, 1931, when she was placed on a synthetic diet low in protein (Cargill) with 0.3

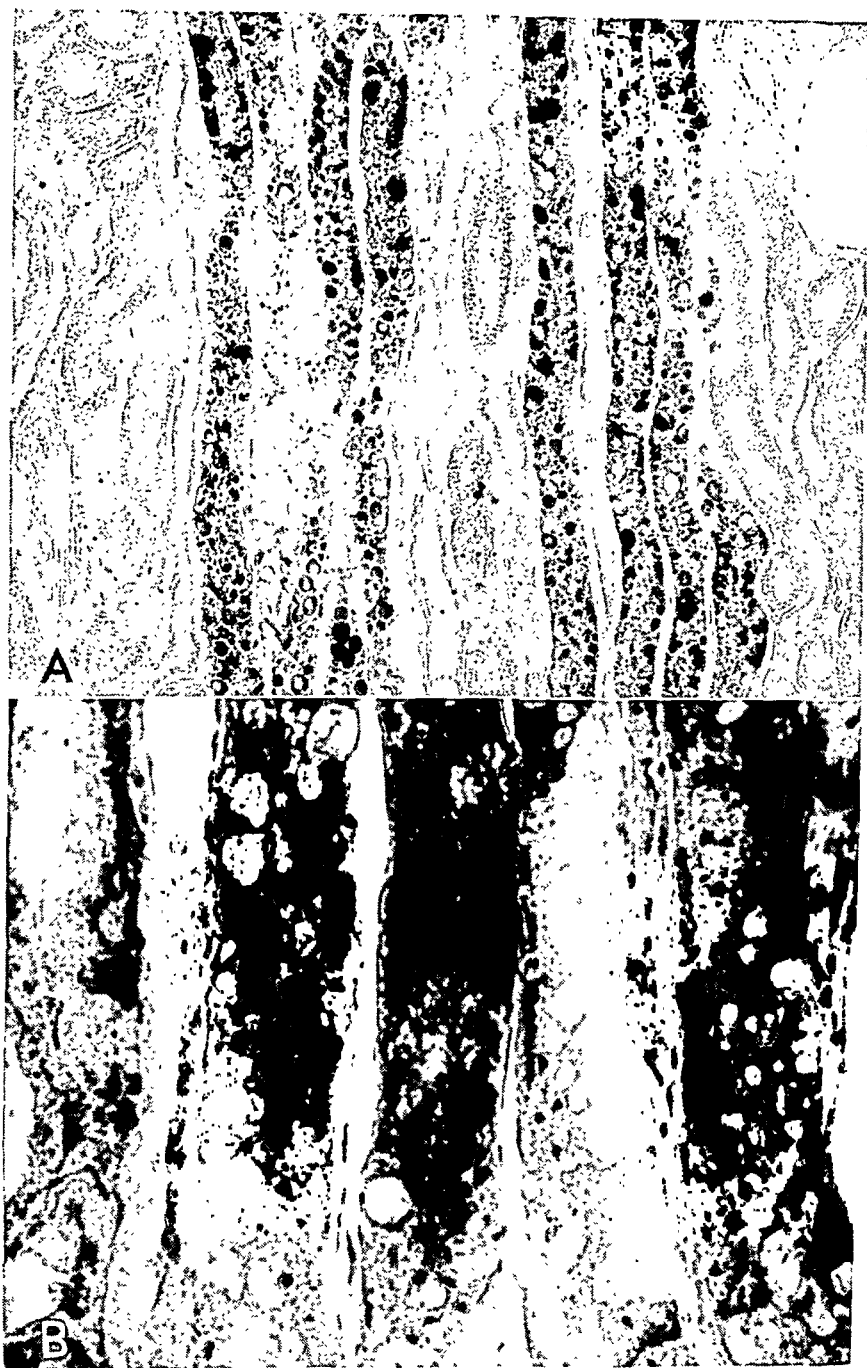
TABLE 3.—*Results in the Case of Dog 2*

Date	Weight, Kg.	Hematoerit, per Cent	Total Plasma Protein, Gm.	Plasma Albumin, Gm.	Plasma Globulin, Gm.	Fatty Acids, Gm.	Cholesterol, Gm.	Serum Phosphorus, Mg.	Blood Urea Nitrogen, Mg.	Diet	Comment
1/23/31	18.5	..	...	...	...	.....	.....	...	..	N	
1/23/31	18.5	53	6.7	5.2	1.5	0.165	0.190	3.5	14	LP	
2/24/31	15.3	..	...	...	...	.....	.....	...	..	A	
3/ 2/31	14.5	49	4.7	2.6	2.1	0.320	0.185	...	..	A	
3/27/31	13.3	38	4.5	2.4	2.1	0.290	0.250	...	..	A	
4/25/31	12.5	39	4.8	2.4	2.4	0.390	0.226	...	..	A	
5/20/31	11.5	34	3.7	2.0	1.7	.....	.....	...	..	A	
6/ 8/31	10.7	31	4.4	1.6	2.8	.....	0.284	...	4	A	1,000 cc. of 1 per cent solution of sodium chloride on 5 successive days
6/12/31	11.0	..	...	...	...	.....	.....	...	..	..	No edema
6/16/31	11.1	30	3.5	2.0	1.5	.....	.....	2.0	..	M	No edema; left nephrectomy
7/ 3/31	11.1	20	3.5	...	...	0.595	0.169	...	4	M	250 cc. of blood withdrawn
7/13/31	11.2	21	3.6	...	...	0.530	0.152	...	4	..	Autopsy

Gm. of protein per kilogram of body weight; this was continued until February 18. The dog was then fed diet A, described in the first paper of this series. We tried various forms of Cargill diets, but we found that after varying periods the dogs refused it, but they ate the vegetable diet (diet A) much better, though as a rule not all of it.

The total plasma protein had fallen to 4.6 Gm., with an albumin-globulin ratio of 3.0:1.5 by the eighty-sixth day. We then administered by gavage 1,000 cc. of a 1 per cent solution of sodium chloride on two successive days, but no edema was observed and there was no gain in weight. However, on the one hundred and fortieth day the total protein had dropped to 3.6 Gm., with an albumin-globulin ratio of 2.3:1.4; the administration of the same amount of salt solution produced extensive subcutaneous pitting edema and a gain in weight of 1.7 Kg. Nephrectomy was performed on the left side on the one hundred and forty-seventh day while the dog was still edematous. The dog was given a transfusion of 450 cc. of whole blood and was then fed a diet of milk and meat with a generous allowance of protein. However, after two days the plasma protein level had not changed

and the edema persisted. Edema disappeared on the fourth postoperative day, and on the sixth day the plasma protein had risen to 5.8 Gm., with an albumin-globulin ratio of 2.8:3.0.



Fatty changes in the renal tubules; sudan III stain. *A*, low power view; *B*, higher power view.

As we pointed out before, anemia gradually develops in animals fed this diet. We determined to control the effect of this factor on the fat deposit in the kidney by maintaining the anemia after the unilateral nephrectomy and allowed the plasma

protein levels to rise and then compared the pathologic picture of the remaining kidney with that of the one removed at operation. We were successful in maintaining the anemia by the withdrawal of large amounts of blood, but the expected rise in plasma protein levels did not occur. Doubtless the failure of the dog to eat all her food was a large factor in this disappointing result. Autopsy was performed on the forty-ninth postoperative day.

Autopsy showed, on external appearance, a marked emaciation. The muscles were pale, and the subcutaneous fat was almost absent. The left kidney was absent. The right kidney weighed 70 Gm. The capsule stripped with ease, leaving a smooth reddish-gray surface. The parenchyma of the kidney was of the usual consistency, and on section there was some yellow striation at the cortical medullary junction. Microscopic sections of the liver revealed a few large vacuoles within the cells throughout the lobule. The cytoplasm was coarsely granular and swollen. The sections of the kidney showed a large amount of fat, largely confined to the cells of the loops of Henle, and most of it stained red or lilac with Nile blue sulphate. There was no doubly refractile lipoid in the especially prepared sections. There was no essential difference in the histologic picture in the right and the left kidney.

Dog 2.—This dog was an adult female gray police dog, weighing 18.5 Kg. She was fed a series of diets similar to those given to dog 1. On the fifty-fifth day, the total plasma protein was 4.5 Gm., with an albumin-globulin ratio of 2.4:2.1. At this time 1,000 cc. of salt solution was administered by gavage, but there was no gain in weight, and no edema appeared. This was repeated on the one hundred and ninth day, when the total protein was 3.7 Gm. and the albumin-globulin ratio 2.0:1.7, and again there was no gain in weight. On the one hundred and thirty-sixth day, the total plasma protein fell to 3.5 Gm., with an albumin-globulin ratio of 2.5:1.0, and again the administration of sodium chloride produced no edema.

Nephrectomy was done on the left on the one hundred and thirty-sixth day. The postoperative diet was the same as that given dog 2, and we again maintained anemia by the withdrawal of large amounts of blood. This dog also showed constantly low plasma protein levels after the operation. She was killed, and autopsy was performed on the twenty-ninth postoperative day. The autopsy report was essentially the same as that of dog 1.

Dog 3.—This was an adult, brown, female police dog, weighing 15.3 Kg. She was fed in the same way as dog 1. The total plasma proteins fell to 4.5 Gm., with an albumin-globulin ratio of 2.5:2.0 on the fifty-fifth day of the low protein feeding. At this time 1,000 cc. of a 1 per cent solution of sodium chloride was administered by gavage on two successive days, but no edema appeared. However, on the one hundred and ninth day the plasma protein had fallen to 3.7 Gm., with an albumin-globulin ratio of 2.2:1.5. At this time the same amount of salt solution was given, and extensive pitting edema developed with a total gain in weight of 1.7 Kg. This dog was so weak that the experiment had to be terminated on the one hundred and twenty-second day, when she was killed and autopsy was performed.

Autopsy showed nothing remarkable in the external appearance except moderate edema at the extremities, which also involved the subcutaneous tissues of the abdomen and thorax. The peritoneal cavity contained 75 cc. of clear light yellow fluid. There was edema of the walls of the stomach, and near the pylorus there were five small ulcers with indurated edges.

The kidneys were similar to those of dog 1, except that near the cortical medullary junction and extending from it into the cortex for a distance of 3 mm.

there was a radial deposition of yellow flecks in the tissue. The microscopic picture was similar to that in dog 1.

Dog 4.—This dog was an adult brown and black female weighing 18 Kg.; it was fed the same series of diets given to dog 2. On the eighty-third day the plasma protein had dropped to 4.9 Gm., and the albumin-globulin ratio was 2.9:2.0. During this time she was getting 1,000 cc. of a 1 per cent solution of sodium

TABLE 4.—*Results in the Case of Dog 3*

Date	Weight, Kg.	Hematoerit, per Cent	Total Plasma Protein, Gm.	Plasma Albumin, Gm.	Plasma Globulin, Gm.	Fatty Acids, Gm.	Cholesterol, Gm.	Serum Phosphorus, Mg.	Blood Urea Nitrogen, Mg.	Diet	Comment
1/23/31	15.3	..	..	..	..	.....	.....	..	..	N	
2/ 1/31	15.7	46	6.2	4.6	1.6	0.365	0.165	4.5	12	LP	
3/ 2/31	13.4	46	4.6	2.5	2.1	0.445	0.232	..	..	A	
3/27/31	11.7	39	4.5	2.5	2.0	0.350	0.199	..	..	A	
4/25/31	10.6	41	4.2	3.0	1.2	0.365	0.185	..	..	A	
5/20/31	9.3	28	3.7	2.2	1.5	.....	.....	..	..	A	
5/22/31	9.3	..	..	..	..	.....	.....	..	..	A	1,000 cc. of 1 per cent solution of sodium chloride on 2 successive days
5/26/31	11.1	20	3.2	1.4	1.8	.....	0.209	3.0	..	A	Edema
5/28/31	11.0	..	..	..	..	.....	.....	..	..	..	Edema
6/ 6/31	9.5	..	..	..	..	.....	.....	..	..	..	No edema; killed and autopsy performed

TABLE 5.—*Results in the Case of Dog 4*

Date	Weight, Kg.	Hematoerit, per Cent	Total Plasma Protein, Gm.	Plasma Albumin, Gm.	Plasma Globulin, Gm.	Fatty Acids, Gm.	Cholesterol, Gm.	Serum Phosphorus, Mg.	Blood Urea Nitrogen, Mg.	Diet	Comment
12/22/30	18.4	....	..	..	..	.....	.....	..	..	N	
1/ 3/31	18.0	45.6	7.0	4.5	2.5	0.275	0.145	4.2	17	LP	
2/24/31	12.5	....	..	..	..	.....	.....	..	..	A	
3/ 2/31	12.5	....	..	..	..	.....	.....	..	..	A	
3/27/31	11.3	40.0	4.9	2.9	2.0	0.340	0.200	..	..	A	
4/25/31	10.0	28.0	3.8	2.8	1.0	0.456	0.186	..	..	A	
5/20/31	8.3	33.0	4.2	2.4	1.8	.....	.....	..	..	A	1,000 cc. of 1 per cent solution of sodium chloride for 2 days
5/23/31	9.0	....	..	..	..	.....	.....	..	..	A	No edema
5/26/31	8.1	....	..	..	..	.....	.....	..	..	A	Died; no edema

chloride on two successive days, and there was no gain in weight. By the one hundred and thirty-seventh day, however, the plasma protein had fallen to 4.2 Gm., with an albumin-globulin ratio of 2.4:1.8. At this time the same amount of salt solution was administered, and she gained 0.7 Kg., but there was no demonstrable edema. This dog died on the one hundred and forty-third day.

Autopsy showed essentially the same changes as in dog 1.

Dogs 5 and 6.—These were control animals; they were fed a diet containing the same amount of fat per kilogram as diet A contained but an adequate amount of proteins (2.2 Gm. per kilogram). They consumed the diet well and were fairly healthy throughout with no evidence of anemia, and they gradually

gained weight. There was little or no change in the plasma protein levels. Autopsies were performed on the one hundredth day.

Autopsy revealed nothing of interest. The kidneys were normal in every respect. The sections stained with nile blue sulphate and sudan III showed very little fat. The difference in the amount of fat in these kidneys and in those of the experimental animals was striking.

#### SUMMARY AND CONCLUSION

Hypoproteinemia and edema were produced in two of four dogs fed a diet low in protein for a long time. A critical level of plasma proteins for the formation of edema in the dog is probably 4 Gm. of total protein per hundred cubic centimeters and 2 Gm. of plasma albumin.

The fatty changes in the renal tubules of all our dogs with hypoproteinemia were shown not to be accidental but the result of the experimental procedure. We are unable to prove that these fatty changes are not due to the anemia which invariably complicates these experiments, but we offer evidence that the anemia is not a potent factor. This fat is not the same as that found in the convoluted tubules in patients with the nephrotic syndrome, for in the dogs the fat is not in the form of doubly refractile spherocrystals.

# LIPOID PNEUMONIA

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The rôle which the exogenous introduction of oily substances plays in the etiology of certain pathologic conditions of the lungs has aroused considerable interest in recent years because of the increasing use of a variety of oils in the treatment of diseases such as disturbances of the gastro-intestinal and respiratory tracts and vitamin deficiencies. The lesion produced in the lungs by the action of these oils consists essentially of a diffuse infiltration of the parenchyma with large monocyctic cells, which engulf the oil in an attempt of the organism to dispose of it, together with other inflammatory cell infiltrates, such as plasma, eosinophilic and giant cells. After the inflammatory process subsides and healing takes place, there results local formation of fibrous tissue which replaces those portions of the parenchyma which previously had been the seat of the pneumonic process. Usually an acute inflammatory pneumonic superimposition occurs which causes death. This lesion has been termed lipoid pneumonia.

It has been established that similar pulmonary lesions can be produced in animals by the intratracheal injection of oily substances. Guieysse-Pellissier<sup>1</sup> showed that following such injection of olive oil in dogs or rabbits, the alveoli become filled with monocytes containing oil and many polymorphonuclear neutrophilic and eosinophilic leukocytes. Corper and Fried<sup>2</sup> described similar lesions in the lungs of animals following the use of olive oil, liquid petrolatum and chaulmoogra oil. Segal and Cohen, of this laboratory, confirmed these observations, in 1926, by intratracheal sprays of albolene in rabbits (unpublished experiments). In 1925, Laughlen<sup>3</sup> reported the case of an adult with paralysis of the larynx and vocal cords and the cases of three children

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1. Guieysse-Pellissier, A.: *Compt. rend. Soc. de biol.* **83**:809, 1920.

2. Corper, H. J., and Fried, H.: *J. A. M. A.* **79**:1739, 1922.

3. Laughlen, A. F.: *Am. J. Path.* **1**:407, 1925.

who showed large quantities of oil droplets with pulmonary changes similar to those described by Guieysse-Pellissier in experimental animals. He was also able to reproduce the lesion in the lungs of animals by the intratracheal injection of oily substances. In 1927, Pinkerton<sup>4</sup> reported six cases with findings similar to those reported previously. He observed, too, that the oily substance in the lungs is removed slowly, and that the end-picture of this process is fibrosis accompanied by giant cell formation. More recently, Pierson<sup>5</sup> reported a case of pneumonia in a child caused by aspiration of lipoids and gave a fairly detailed account of the findings in the lungs. He stated that the oil causes a profound and rapid reaction, which represents an effort on the part of the body to expel the foreign material. He expressed the opinion that pneumonia caused by lipoids is not common, although the realization of its possible existence may lead to more frequent diagnosis.

This paper has for its purpose a review of the subject to the present time and a presentation of six typical cases which illustrate this condition.

#### REPORT OF CASES

CASE 1.—A girl, 9 weeks old, was admitted to the hospital on Nov. 29, 1925. Four days before the mother noted that the baby was fretful and in pain: this continued for three days. On the day before admission the patient took her feeding badly, was drowsy and irritable, became cyanotic and had several soft, greenish bowel movements. She was a normal infant, born at full term, had been cyanotic since birth, and had gained only 1 pound (4.5 Kg.).

The patient was very ill with a temperature of 97.2 F.; she was markedly cyanotic, and respiration was of a Cheyne-Stokes character. A brownish-red, mucoid material was expectorated frequently. There were dulness over the bases of both lungs and bronchial breathing. A loud systolic murmur was heard over the precordium. She died within three hours after admission. The red blood cells numbered 3,620,000; the hemoglobin was 75 per cent, and leukocytes numbered 27,000. A blood culture showed *Staphylococcus anhaemolyticus*. The clinical diagnosis was congenital heart disease and bronchopneumonia.

At autopsy the body was that of a poorly nourished infant. The heart was enlarged, and the mitral valve was covered with numerous vegetations. The ductus arteriosus was patent; the large vessels were transposed. The lungs showed a few pleural adhesions on the left side and a small amount of straw-colored fluid in the left pleural cavity. The upper lobe and the posterior portion of the lower lobe of the right lung and the entire posterior portion of the left lung were of a dark bluish color and firm. Microscopically, the discolored and firm portions showed an intense inflammatory process associated with atelectatic changes. The exudate was rather peculiar, as it consisted mainly of large monocytic cells

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4. Pinkerton, H.: *Am. J. Dis. Child.* 33:259, 1927.

5. Pierson, J. W.: *J. A. M. A.* 99:1163, 1932.



which were heavily laden with fat globules. This cellular reaction was apparent everywhere, particularly within the lumens of the alveoli and, in places, within the alveolar walls. Some of the fat globules were extracellular, lying freely in the alveoli and alveolar walls. Associated with this lipoid process was a severe inflammatory reaction characterized by an exudate consisting of polymorphonuclear leukocytes and round cells (fig. 1).

Subsequent information from the mother revealed that the child's throat had been sprayed with an unidentified oily substance for some time.

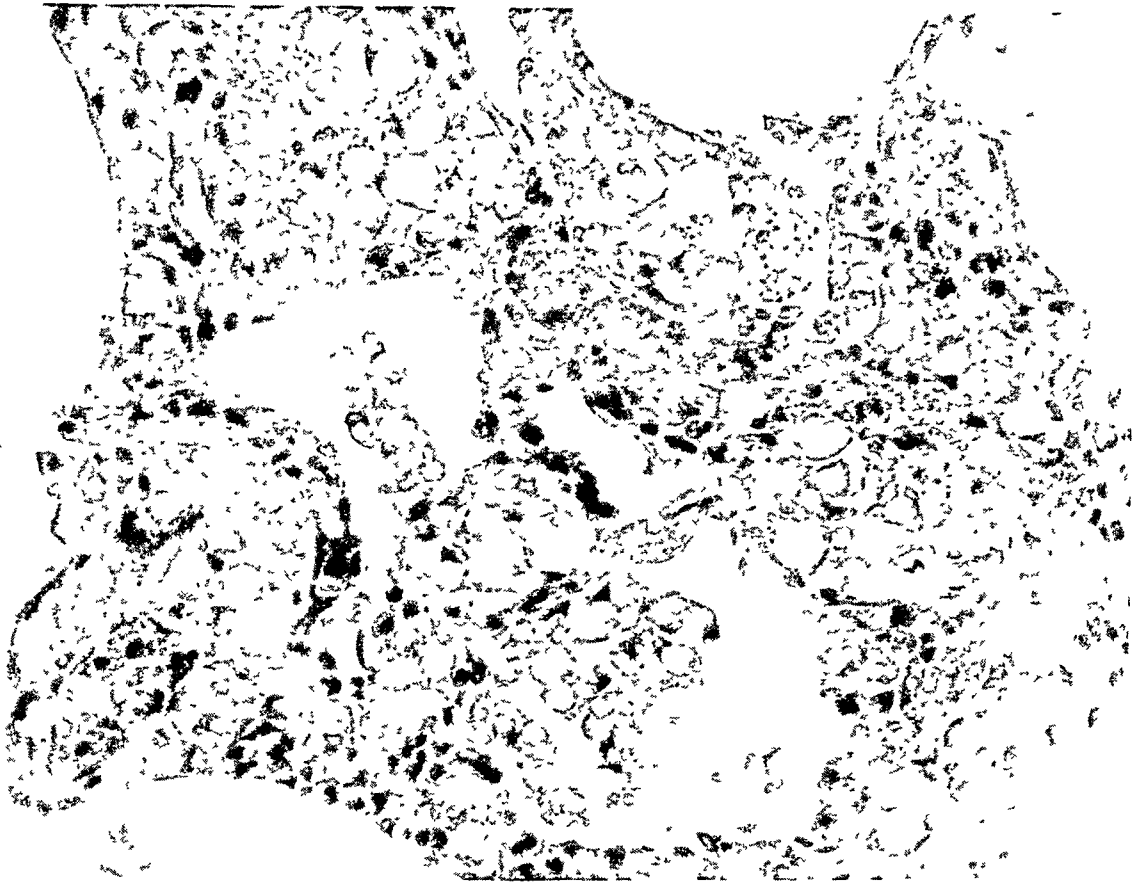


Fig. 1 (case 1).—Fat-laden monocytes within the alveolar spaces and alveolar walls of the lungs.

The following four cases present no history of aspiration of oily substances into the lungs, although postmortem examination showed their presence. In all of the cases, however, there was a history of vomiting, and it is possible that during vomiting small amounts of fat, perhaps from milk, were accidentally aspirated.

CASE 2.—A boy, 6 months of age, was admitted to the hospital on Feb. 10, 1933, because of vomiting and diarrhea. He was apparently well until eight days before, when he had diarrhea with blood and mucus in the stools. His temperature was

104 F. two days before entrance and later fluctuated between 99 and 101 F. He was well developed and nourished. Respirations were slow and labored; the breath had an acetone odor. The lungs were normal on percussion and auscultation. The child died on the day following admission.

At autopsy, the lungs showed diffuse areas of consolidation. Microscopic examination of these areas revealed numerous large monocytic cells within the alveolar spaces. The cytoplasm of these cells was largely replaced by fat vacuoles which caused the cell to appear as a pale structure, while the nucleus was displaced toward the periphery. Other portions of the lung showed an acute pneumonic process with dense leukocytic infiltrations.

CASE 3.—A girl, 9 months old, was admitted to the hospital on Dec. 2, 1932, because of convulsions, underdevelopment and poor feeding. She showed evidence of an infection of the upper respiratory tract and of hypoglycemia. The liver was large, reaching to the brim of the pelvis. The temperature rose to 106 F., and the patient died on the day of admission.

At autopsy, the lungs showed diffuse areas of bronchopneumonia. Microscopically, many of the alveolar spaces in these areas were infiltrated with an abundance of large cells, the cytoplasm of which was replaced by multiple tiny fat vacuoles. In other areas the alveolar exudate consisted of large collections of polymorphonuclear leukocytes. Incidentally, the liver showed marked fatty changes, and the islands of Langerhans were markedly hypertrophied.

CASE 4.—A boy, 3 months of age, was admitted to the hospital on March 16, 1933, because of vomiting, fever and diarrhea. Two weeks before admission he had a temperature of 103 F., which later fluctuated between 102 and 106 F. He took his feedings poorly, vomited several times a day and later showed diarrhea. On admission he was acutely ill, drowsy and breathing rapidly. The tonsils and throat were deeply congested; he had bilateral otitis media. A few hours after admission he died.

At autopsy, there were found in the lungs patchy areas of consolidation, in which the alveoli and bronchioles showed microscopically a purulent exudate. Many of the alveolar spaces and bronchioles contained numerous large monocytic cells, the cytoplasm of which contained many small fat globules. Some of these cells were found within the alveolar walls and in the adjacent lymph spaces.

CASE 5.—A boy, 15 months of age, was admitted to the hospital on March 11, 1933, because of projectile vomiting, constipation and underweight. He was apparently well until the age of 5 months, when he began to have attacks of vomiting, which continued until admission. He was fairly well developed, irritable and apathetic. The lips were slightly cyanotic. The fingers and toes were clubbed. The heart and lungs were recorded as normal. Bronchopneumonia developed, and the patient died on April 3. The important observations at autopsy were confined to the kidneys and lungs, the former being the seat of an acute and subacute glomerulonephritis.

The lungs showed pleural adhesions at the apexes. In this region the pulmonary tissue was firm and consolidated. The rest of the lungs showed no significant changes. Sections from the apexes showed numerous large, pale cells within the alveoli. Many of these cells were filled with multiple tiny fat vacuoles.

The septums also contained many of these cells, as well as homogeneous globular bodies. Similar cells were found in the lymph spaces. Other patches showed the usual pneumonic changes.

The following case is of particular interest and therefore will be described in greater detail. It concerns a 4 year old child who suffered from encephalitis and bulbar paralysis and in whom a pneumonic process developed as the result of the long-continued administration of large amounts of cod liver oil.

CASE 6.—A boy, 4 years old, was admitted to the hospital on Nov. 14, 1931, because of inability to walk for the past five months. Following whooping cough at the age of 2 years, there developed symptoms indicative of encephalitis, with a high temperature, convulsions, loss of consciousness, cyanosis, frothing at the mouth and incontinence of urine and feces. He showed marked improvement during his stay in the hospital. He was readmitted, however, on Oct. 28, 1932, with symptoms of laryngeal paralysis and difficulty in talking and walking. On examination, there was evident a generalized spasticity of the extremities and of the back. All deep reflexes were exaggerated; the facial expression was fixed, and there was no display of emotion except when the child was crying. During his stay in the hospital, the temperature ranged between 99 and 100 F. until December 31, when encephalography was performed; 105 cc. of spinal fluid was removed, and 100 cc. of air was injected. On the following day he became stuporous and cyanotic; he had marked general convulsions and finally died.

At autopsy, the body was fairly well developed and well nourished. The lungs were voluminous and free from adhesions. The pleural surface was mottled with areas of pinkish-yellow discoloration which shaded into deep red toward the base. There were noted externally discrete purple-red nodules which were sharply demarcated from the surrounding lighter-colored surface and measured, on the average, from 5 to 6 mm. in diameter. These nodules felt firmer than the remainder of the lung and resembled small infarcts which had become impregnated with carbon. The cut surface of these nodules was distinctly firmer and drier than the surrounding parenchyma, while that of the remaining lung showed a diffuse purple-red discoloration and a moderately firm consistency. The bronchioles were considerably thickened and dilated and contained pus, mucus and frothy fluid. In the apical region of the right lung was a circumscribed abscess about 2 cm. in diameter, surrounded by a well formed pyogenic membrane and containing necrotic material mixed with thick yellow pus.

Microscopic examination of the lungs showed the essential lesion to be an inflammatory process in which large mononuclear cells formed the bulk of the exudate. These cells filled the alveoli and contained many fatty particles. The latter appeared as small spherical bodies, occupying the larger portion of the cell cytoplasm, with displacement of the nucleus toward the periphery. Large numbers of fat globules were also found in the alveoli and in the septums. Frozen sections showed these globules to be a fatty substance which stained bright red with scarlet red. The areas corresponding to the nodules described in the gross

showed local necrosis with an intense inflammatory reaction. The cells consisted chiefly of monocytes filled with fat droplets and to a lesser degree of polymorphonuclear leukocytes and plasma cells. The unaffected alveoli adjoining the pneumonic areas showed emphysema. The exudate in the abscess consisted mostly of leukocytes and to a smaller extent of fat-laden monocytes. Toward the periphery there were granulation tissue rich in capillaries and a fibroblastic process. Extracellular and intracellular oil droplets could be seen in fairly large numbers. Another striking feature in the lungs was peculiar nodular formations of concentric rings of epithelial, giant and fibroblastic cells, in many instances encysting

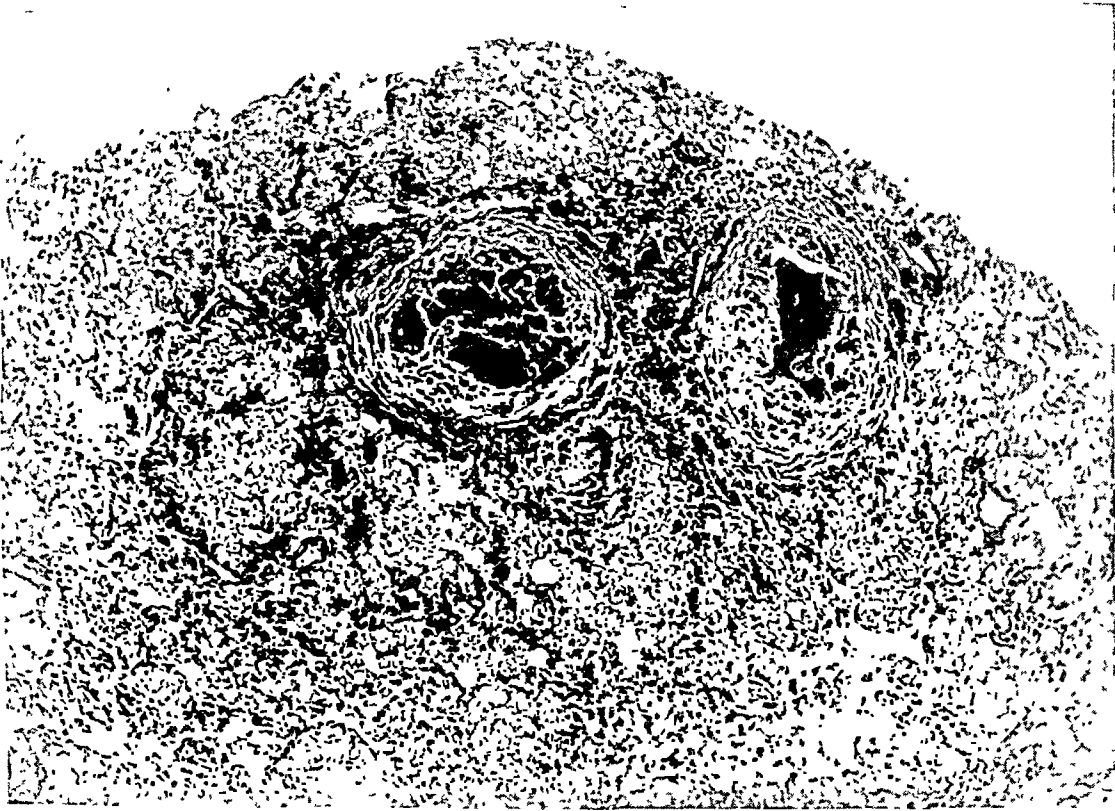


Fig. 2 (case 6).—Granulomatous areas in the lung, consisting of concentric rings of fibroblastic tissue, epithelial cells and scattered giant cells. In the midst of these are oil droplets.

in their midst fat vacuoles. The general appearance resembled tubercles. However, the epithelial cells which formed the most conspicuous part of the lesion simulated more the lining epithelium of the bronchioles. These lesions were interpreted as attempts to encyst the foreign material, similar in nature to granulomas caused by the presence of foreign bodies (figs. 2 and 3). The tracheobronchial lymph nodes contained fat-laden cells but were otherwise normal. In the brain, the left lateral ventricle was markedly dilated. Microscopically a widespread gliosis was noted, particularly in foci around the ventricular system. Microglia, especially the protoplasmic glia, were considerably increased.

The microscopic changes in the lungs of this patient were similar to those described by other authors. The formation of granuloma-like lesions typified a process peculiar to foreign body tissue reactions. The mode of entry of the foreign oily substance into the lungs is interesting. The mother stated that she had forced large quantities of cod liver oil

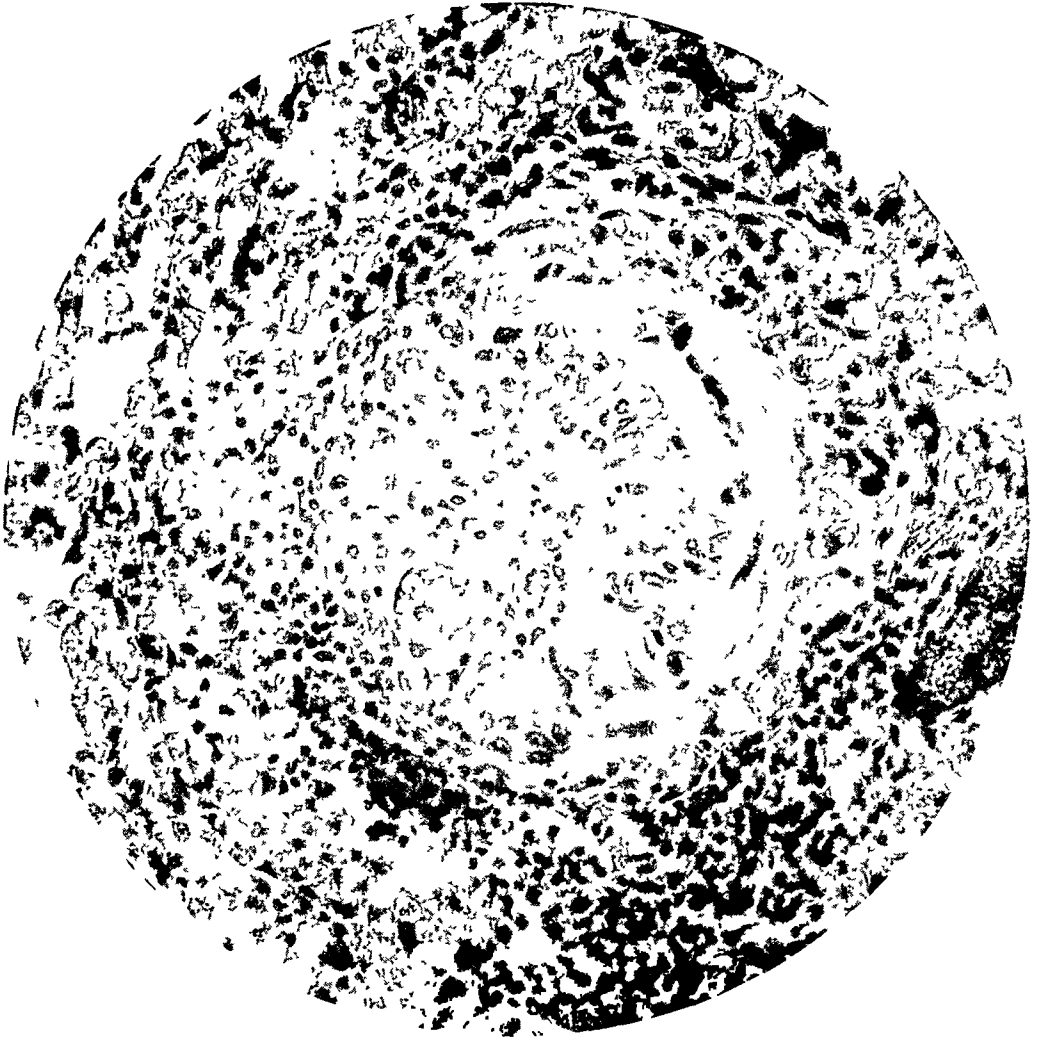


Fig. 3 (case 6).—High power photomicrograph of one of the granulomas, showing the presence of the oil-laden monocytes within this structure.

into the throat of the child for a long time in an attempt to improve his general health. It is probable that the child, already afflicted with encephalitis and bulbar paralysis, had lost the protective mechanism of preventing foreign matter from being aspirated into the lungs. As a result of this defect the oil apparently found its way into the lungs and was the cause of the lipoid pneumonia.

## COMMENT

The appearances in these cases are striking and indicate certain significant conclusions. The pulmonary reaction to the oily material is much the same as that to any other foreign body. It consists of an inflammation which has for its main purpose, apparently, the ridding of the organism of the oily material. The cellular exudate in the alveoli and bronchioles consists for the most part of large monocytic cells which engulf the oil droplets and transport them to the local lymph channels and the regional lymph nodes. The chief interest centers, therefore, about the large monocytes, while the other cells which participate in the picture are of secondary importance. The latter cells are polymorphonuclear neutrophilic and eosinophilic leukocytes and plasma and giant cells. The fibrous tissue formation later in the disease is the end-result of the inflammatory lesion and represents an attempt at healing by scar tissue.

The formation of granulomas is of particular interest as a localized reaction to the oily substance, which results in the creation of concentric layers of epithelial cells, giant cells and fibroblastic tissue encysting droplets of oil. Thus, the foreign body substance is disposed of in at least three different ways: (1) expectorated with the monocytes, (2) taken up by the lymphatics or (3) encysted in the granuloma. These three ways of eliminating the oily substance may be seen in the same lung, although at times one process may predominate.

That the amount of oil and the length of time of administration have a definite bearing on the pulmonary lesions there can be no doubt. Moreover, that the chemical composition of the oil may have some influence on the pulmonary reaction has been suggested by some. If the quantity of oil which finds its way into the lungs is abundant and the administration is continued over a long time, the inflammatory reaction will be more intense and widespread. It has been shown, both clinically and experimentally in animals, that within a few minutes after the introduction of oil into the lungs there occur an inflammatory reaction and an invasion by large monocytes. The elimination of the oily substance, on the other hand, is slow. Since the introduction of iodized poppy seed oil as a diagnostic aid, cases of lipoid pneumonia due to this substance have been observed. One of us (J. R.) recently saw such a case following the intratracheal introduction of this substance in an old person who later died of bronchopneumonia. The lungs showed changes similar to those seen in lipoid pneumonia. The frequent use of oils in pediatric practice by way of the mouth and nasopharynx offers ample opportunity for the oil, especially in the very young, debilitated child, to find its way into the lungs.

Clinical and experimental evidence indicates that areas of lipoid pneumonia may occur without symptoms and signs in most cases.

The present study indicates the need for a change from the treatment of children by means of oily substances administered via the nasopharynx. It is evident that pulmonary inflammations due to the aspiration of lipoids are not detected often enough clinically. This is mainly because the initial lesion in the lungs is not sufficiently widespread to yield detectable clinical or roentgenologic signs. The diagnosis may be made, however, if a history of administration of an oily substance by way of the respiratory tract is obtained. It is also suggested that a careful examination of the expectorated material for monocytes containing oil droplets might be of value. Finally, serious pulmonary complications might be avoided by the more careful and controlled use and selection of oily substances for treatment by way of the nasopharynx, especially in young, debilitated children.

#### SUMMARY

Lipoid pneumonia occurs most frequently in infants. When it occurs in old persons it is usually due to laryngeal paralysis or to direct intratracheal introduction of oil.

The anatomic changes produced in the lungs by oil are characteristic and consist essentially of large monocytic cell infiltration and granulomatous formation. The greater portion of the oil is engulfed by monocytes and is either expectorated or carried to the regional lymphatics. Part of it is encysted in granulomatous tissue.

It is suggested that the diagnosis of lipoid pneumonia can be made in a certain number of cases by the examination of expectorated material for oily substances in the monocytic cells.

# HISTOLOGY OF CERTAIN ORGANS AND TEETH IN CHRONIC TOXICOSIS DUE TO FLUORINE

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AND

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Studies on the growth and reproduction of rats receiving fluorides constantly as a part of the diet have recently been reported.<sup>1</sup> Large numbers of animals were used, and a systematic effort was made to determine the mechanism of the observed toxic effects by means of histologic examinations of all the principal organs. Some of the results of this survey are presented here.

The amounts of fluorine given to these animals were generally below the levels causing acute symptoms of toxicosis but were such as to produce evidence of chronic poisoning. The amounts used were between 15 and 30 mg. of fluorine per kilogram of body weight daily.

Reports in the literature on the effects of the ingestion of fluorine in comparable amounts exhibit variable results. Sollmann<sup>2</sup> stated that no histologic changes were produced by sodium fluoride when fed in amounts of from 50 to 150 mg. per kilogram of body weight per day. Kick<sup>3</sup> reported that the feeding of sodium fluoride had no effect on the liver, kidney, spleen, thyroid or parathyroid of rats. He found that 1 per cent of rock phosphate did not cause degeneration of the epithelium of the convoluted tubules in the kidney of the rat. Hauck, Steenbock and Parsons<sup>4</sup> could detect no histologic changes in eighty-eight parathyroid glands, sectioned serially, from rats fed 0.15 per cent sodium chloride. They mentioned degenerative changes in the kidney

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From the Department of Agricultural Chemistry, University of Wisconsin.

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and testes. Cristiani<sup>5</sup> studied the histologic changes in the hypophysis cerebri and the thyroid glands of guinea-pigs suffering from fluorosis. Macroscopically, the hypophysis was reported to be diminished in size and volume. Microscopically, the reduction in size of the gland was due to atrophy of the cellular elements. The connective tissue stroma was distended and the blood vessels showed dilation which gave the appearance of chronic passive congestion to the gland. The acinal cells were reported to have become smaller in size and poor in cytoplasm which was remarkably free of granular material. Small amounts of sodium fluoride caused a proliferation of the parenchymatous tissue of the thyroid gland in 14 experimental animals. Chaneles<sup>6</sup> studied the histologic changes in rats fed a diet of milk and bread plus 50 mg. of sodium fluoride per kilogram of body weight per day. His examination of the thyroid gland, testes, suprarenal glands, heart, spleen, liver, kidney and bone yielded negative results except for slight congestion in the liver, spleen and suprarenal glands.

Goldenberg<sup>7</sup> reported that a daily intake of from 2 to 3 mg. of sodium fluoride caused hypertrophy of the thyroid gland in the rat, and that sodium fluoride administered either orally or intravenously ameliorated the clinical symptoms of exophthalmic goiter. Tolle and Maynard,<sup>8</sup> however, found no differences in the weight of the thyroid gland between controls and fluorine-fed rats.

#### EXPERIMENTAL METHOD

Tissues were taken for histologic examination from the animals used in the studies previously described.<sup>1</sup> In all, 1,798 preparations representing more than 300 animals were made. The tissues thus obtained represent experimental animals fed various amounts of sodium fluoride or rock phosphate and their controls. The basal diet, which is given in detail in the papers cited, contained yellow corn, wheat middlings, linseed oil meal, alfalfa meal, tankage, ground limestone, bone meal and sodium chloride. The rock phosphate contained 3.52 per cent fluorine. The additions to the basal diet were given under a variety of experimental procedures, but in most cases they were given throughout the life of the animal. In groups A-1, A-3 and A-7 second, third, fourth and fifth generation rats are included.

The tissues taken for study were: liver, kidney, thyroid, suprarenal, hypophysis, ovary, testes, femur and incisor teeth. These tissues were fixed in the

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5. Cristiani, H.: *Compt. rend. Soc. de biol.* **103**:556, 745 and 981, 1930; **107**:554, 1931.

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7. Goldenberg, L.: *Compt. rend. Soc. de biol.* **95**:1169, 1926; *Semana méd.* **2**:1659, 1932.

8. Tolle, C., and Maynard, L. A.: *Cornell University Agricultural Experiment Station, Bull.* 530, 1931.

usual manner in a trinitrophenol-formaldehyde solution<sup>9</sup> or in Zenker's fluid to which a dilute solution of formaldehyde was added; they were dehydrated in graded alcohol and embedded in paraffin. In the case of the bones and the teeth, decalcification was effected by means of 5 per cent nitric acid in 70 per cent alcohol during the dehydration process. A few teeth were fixed, dehydrated, embedded in balsam and ground thin for examination of the enamel layer. All sections were stained with hematoxylin and eosin in the usual manner.

## RESULTS

The microscopic appearance of the tissues was quite normal in comparison with the severe gross symptoms of chronic fluorosis. At the moderate levels used in the greater part of these experiments the glands and organs concerned with reproduction were unaffected. The high level of 0.30 per cent sodium fluoride caused marked degenerative changes in the testes. The ovaries, hypophysis and suprarenal glands were in most cases normal, although fatty degeneration was noted in the suprarenal glands in a few cases and there was a tendency toward passive congestion of this gland as indicated by the distended appearance of the blood vessels and sinusoids, particularly in the region of the zona reticularis of the medulla.

The deviations from the normal histologic appearance most frequently observed in the soft tissues were in the kidney and thyroid gland. Frequently ecchymotic or petechial hemorrhages occurred which might be taken as evidence of toxemia. These hemorrhages occurred in the endocardium and particularly in the mucosa of the pylorus. Hemorrhages of the pylorus in fluorine-fed rats were also observed by Hauck and her co-workers.<sup>4</sup>

The deviation most often noted in the thyroid gland was a change in the epithelial elements (fig. 1). The normal uniformly low cuboidal cells lining the acinus changed into a low columnar type of cell resembling a parenchymatous proliferation. The changes noted were not extensive, and in most cases a portion of the gland retained its normal appearance. The character of the colloidal material was less frequently changed. There seemed to be a tendency toward more desquamation of the epithelial cells in the animals receiving fluorine. High levels of fluorine intake over short periods of time failed to produce these changes. There was some evidence of occasional fibrosis.

The kidney was most frequently affected. The nature of the changes were usually those of parenchymatous degeneration in the convoluted portion of the renal tubules, and occasionally hyaline degeneration in the blood vessels (fig. 2). In acute cases the tubules became greatly distended. Macroscopically, the kidney became spotted.

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9. Solution of formaldehyde, U. S. P., 500 cc.; distilled water, 1,875 cc.; glacial acetic acid, 125 cc.; trinitrophenol to saturation point.

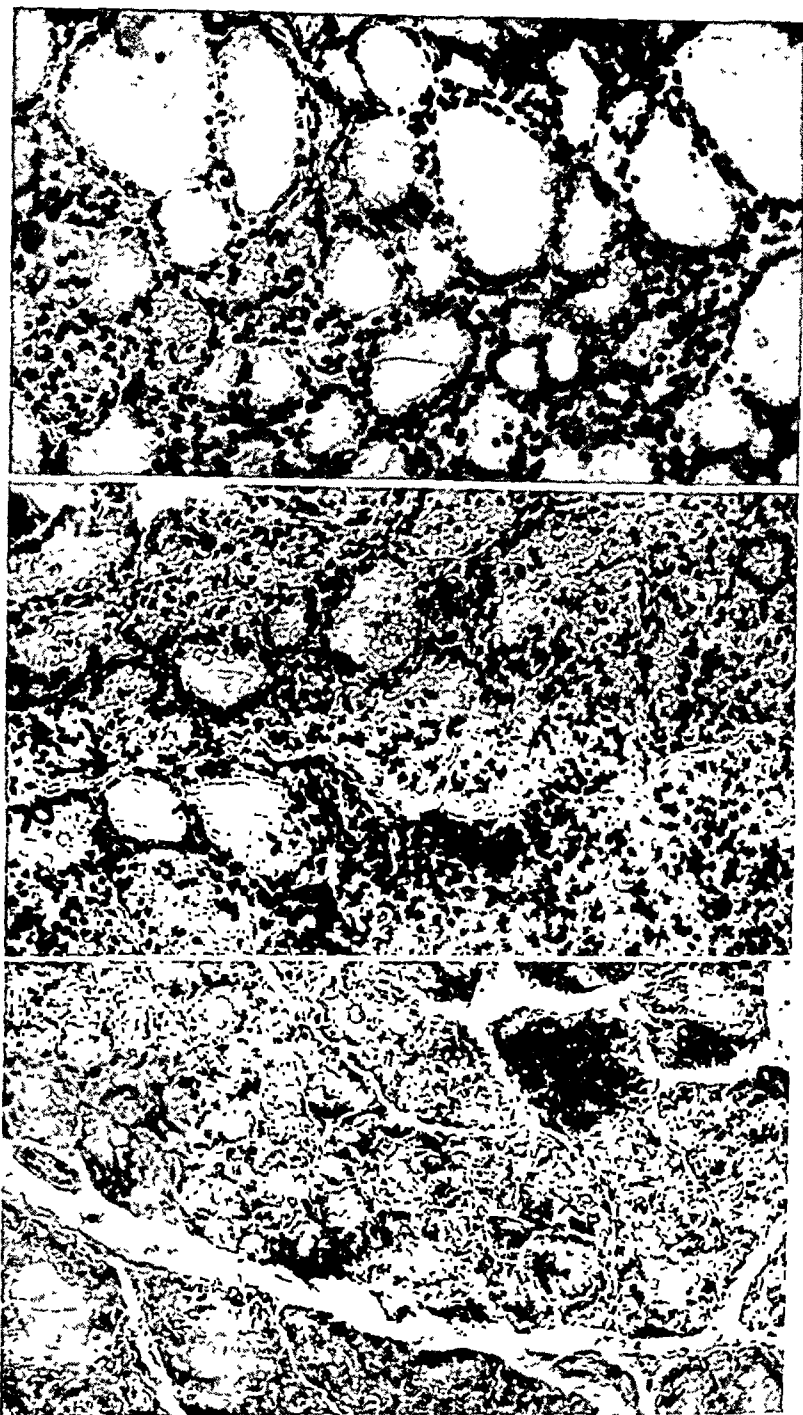


Fig. 1.—The thyroid gland: 1, thyroid gland from a normal rat fed the basal A-1 ration;  $\times 500$ . 2, thyroid gland from a rat fed the basal A-1 ration plus 1 per cent rock phosphate which contained 3.5 per cent fluorine. Cellular infiltration and fibrosis are evident in this portion of the gland;  $\times 500$ . 3, thyroid gland from a rat fed the basal A-1 ration plus 0.043 per cent sodium fluoride. The acinar cell is distinctly transformed into a parenchymatous proliferative type of cell. The presence of numerous large cells with transparent cytoplasm is apparent (a). These acini in this gland suggest changes caused by fasting;  $\times 500$ .

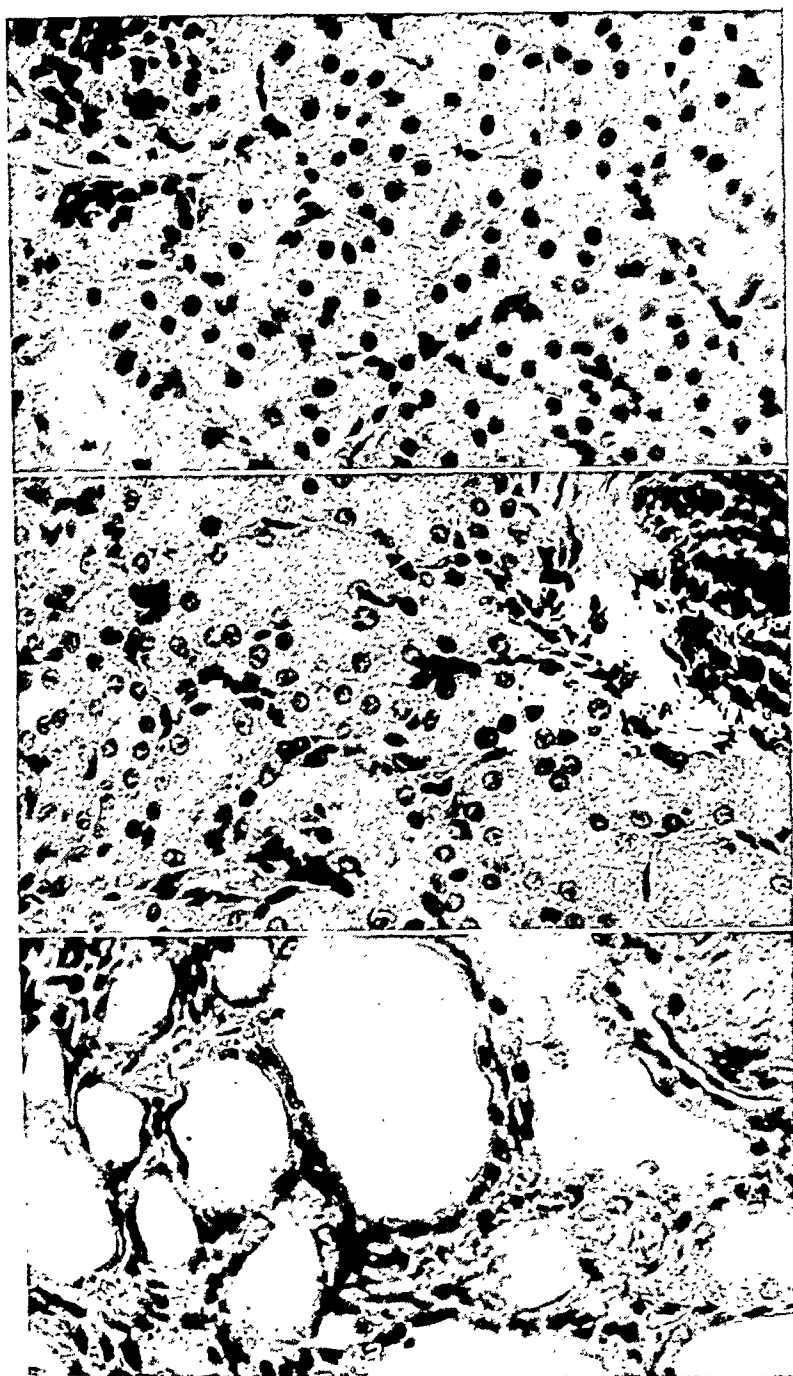


Fig. 2.—The kidney: 1, kidney from a rat fed the basal A-1 ration;  $\times 500$ . 2, kidney from a rat fed the basal A-1 ration plus 1 per cent rock phosphate which contained 3.5 per cent fluorine. The disappearance of occasional nuclei and the occurrence of pyknosis indicate degenerative changes in this portion of the kidney. A hemorrhagic area is present;  $\times 500$ . 3, kidney from a rat fed the basal A-1 ration plus 0.15 per cent sodium fluoride. This represents the severe effects of fluorine on the kidney. It is marked by considerable dilatation and distention of the renal tubules. In most cases much cellular debris is found in the lumen of the tubules, and occasionally renal casts are seen;  $\times 500$ .

The table indicates that the testes, ovaries and liver were also affected to some degree. Except at the higher levels of fluorine intake, the degeneration of the testes may have been influenced by senility, as the only male showing testicular degeneration at a low fluorine intake was approximately 15 months old, and this change is not infrequently noted in control animals at this age. In the A-3 and A-7 lots the females showing changes in the ovaries had all produced one litter each and subsequently failed to become pregnant. In these cases persistent corpora lutea were present and few or no developing follicles were observed. The changes observed in the liver were those of fatty degen-

*Summary of the Frequency of the Occurrence of Histologic Changes in Soft Tissues Noted in Animals on Fluorine Diets as Compared with Controls*

Tissue	A-1 or Basal Ratio			A-3 or Basal Plus 0.043% Sodium Fluoride			A-6 or Basal Plus 0.6% Rock Phosphate			A-7 or Basal Plus 1% Rock Phosphate		
	No. of Animals	Ab- nor- mal	Per Cent Ab- normal	No. of Animals	Ab- nor- mal	Per Cent Ab- normal	No. of Animals	Ab- nor- mal	Per Cent Ab- normal	No. of Animals	Ab- nor- mal	Per Cent Ab- normal
Liver.....	33	0	0	44	1	2.9	8	0	0	44	1	2.3
Kidney.....	44	6	13.7	49	22	44.9	7	2	28.6	46	14	30.4
Thyroid.....	38	4	10.8	43	19	44.1	5	3	60.0	46	10	21.9
Suprarenal...	39	0	....	43	5	11.6	2	..	0	45	2	4.4
Ovary.....	24	0	....	18	3	16.6	3	..	0	29	3	10.5
Testis.....	9	0	....	9	1	11.1	2	..	0	10	0	....
Hypophysis..	2	0	....	6	1	16.6	..	..	....	..	..	....

	Basal Plus 0.10% Sodium Fluoride			Basal Plus 0.20% Sodium Fluoride			Basal Plus 0.30% Sodium Fluoride		
	No. of Animals	Ab- nor- mal	Per Cent Ab- normal	No. of Animals	Ab- nor- mal	Per Cent Ab- normal	No. of Animals	Ab- nor- mal	Per Cent Ab- normal
Liver.....	3	0	0	4	1	25.0	6	1	16.6
Kidney.....	3	1	33.3	4	1	25.0	6	3	50.0
Ovary.....	2	0	0	1	0	0	2	0	0
Testis.....	2	0	0	3	0	0	3	3	100.0

eration, which occurred in 2 cases, and occasionally intracellular edema was noticed. These changes may or may not have been due to the effects of the fluorine.

A total of 122 decalcified slides prepared from the maxillae and mandibles with cross-sections of the teeth, and also of the radius and ulna of young rats up to 8 weeks of age, showed no variation from normal. The teeth exhibited normal pulp, characteristically arranged odontoblasts, well developed dentinal tubules, and apparently normal calcification of the dentin. Abnormal calcification of the long bones was not detected at this age.

The bleaching effect of fluorine on the teeth of rats has been described in general terms by other workers. It is interesting to observe the development of this phenomenon in mature animals fed our basal ration plus 0.15 per cent sodium fluoride. The bleaching effect is first noticed on the mandibular incisors between the fourteenth and sixteenth

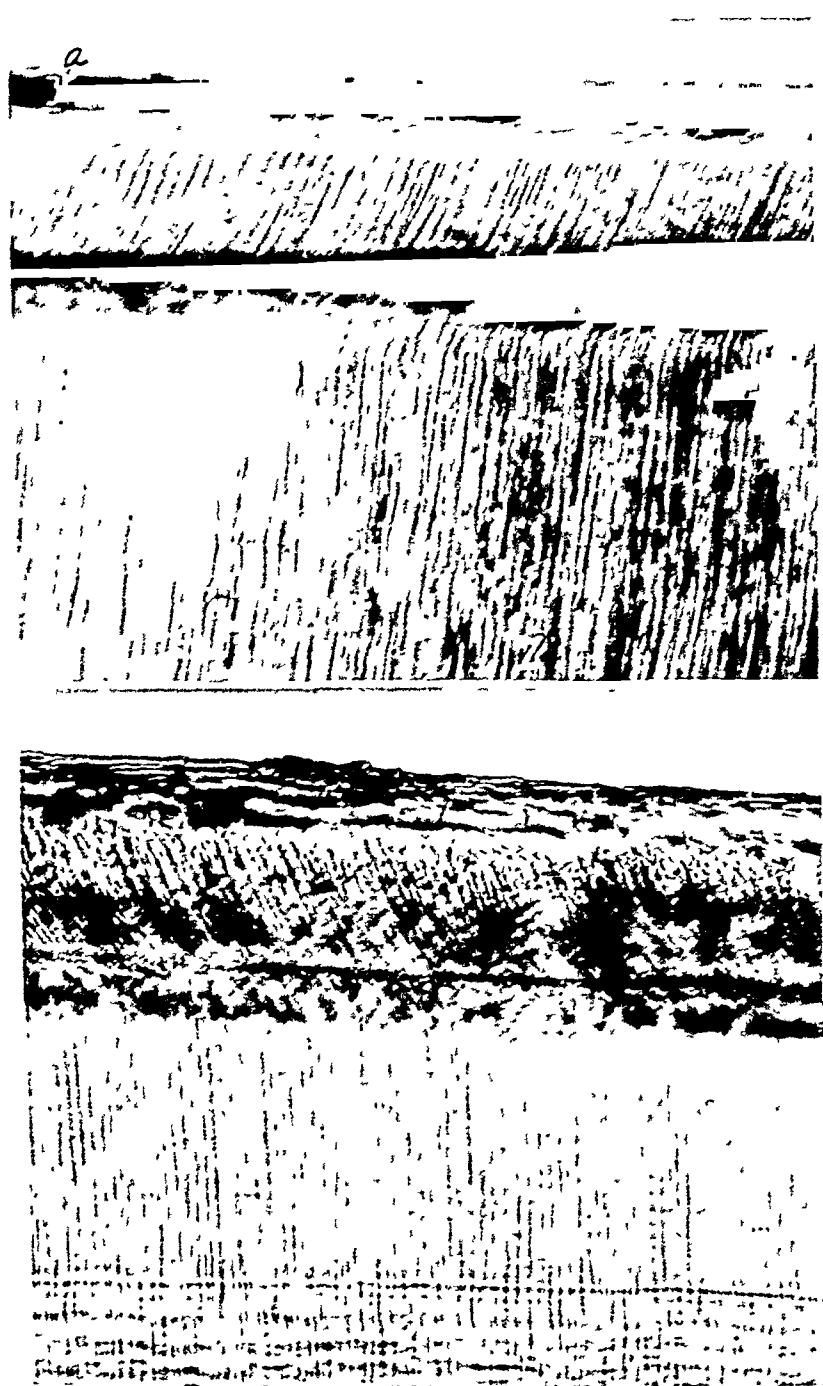


Fig. 3—The teeth: 1, a ground section of a tooth from a rat fed the basal A-1 ration. Note the regular and uniform enamel rods and the outer dark band (*a*) which represents the amber-colored surface of the normal enamel in the rat tooth;  $\times 200$ . 2, a ground section of a tooth from a rat fed the basal A-1 ration plus 0.15 per cent rock phosphate until the amber color was lost, approximately thirty days. This tooth was ground somewhat thinner than the section in 1. Note the "flaky" character and loss of density of the outer portion of the enamel layer;  $\times 200$ .

day after the incorporation of the sodium fluoride in the ration. It is first noticeable as a white chalky band at the base of the teeth. The band widens rapidly, ascending the tooth and causing a change from the normal amber color of the anterior surface of the tooth to a chalky lusterless white. There is a distinct line of demarcation between the two sections of the tooth, and the progress of the decoloration is easily followed. Usually the bleaching of the full length of the tooth requires from ten to twelve days. The mandibular incisors bleach first, followed by the maxillary incisors in the same manner. Both pairs of incisors are thus completely bleached in approximately fifty days. Coincident with the bleaching there is a failure to wear normally, and the usual chisel edge is lost. The teeth become thickened and elongated, with the increase in size due principally to an increase in the dentine.

Ground sections of these teeth present a defective enamel in the outer one third of this structure (fig. 3). While there seems to be a tendency to a more widely dispersed area of Tomes, the chief variation from normal lies in the colored portion of the enamel. The enamel rods appear to be normal in size, length and form, but the outer portion becomes flaky and loses the compact appearance and the color present in the normal tooth. Abnormal calcification appears likely and suggests either defective calcification or removal of calcium. Chipping of this outer layer is noticeable in teeth from rats which have been subjected to a long-continued ingestion of fluorine.

#### SUMMARY AND CONCLUSIONS

Histologic changes have been observed as the direct result of including fluorine in the diet of rats, either in the form of the sodium salt or as rock phosphate. Degenerative changes were found most frequently in the kidneys, with degeneration of the testes at the higher levels of ingestion of fluorine. The degree of deviation from the normal histologic appearance was closely related to the level of the fluorine ingested. The histologic appearance of the hypophysis remained practically normal, which is in harmony with the fact that it maintains its normal weight and functioning power<sup>1b</sup> with respect to gonadal stimulation. No reduction in size of the gland and no atrophy of the cellular elements were observed. A mild parenchymatous proliferation occurred in the thyroid gland when sodium fluoride was fed. No gross hypertrophy of this gland was observed.

These studies of toxicosis due to the ingestion of fluorine indicate that fluorine produces pathologic changes in the kidneys, incisor teeth and thyroid glands in the rat when from 20 to 30 mg. of fluorine per kilogram of body weight is ingested daily. To a lesser and more variable extent, pathologic changes have been noted in the liver and suprarenal glands. Pathologic changes in other tissues were not detected.

# NATURE AND ORIGIN OF THE XANTHOMA CELL

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During the past half century much work has been done and many observations recorded dealing with xanthomatous processes. In this study an attempt has been made to determine the origin and more particularly the nature of the fundamental cell common to these processes—the xanthoma cell or foam cell.

The material studied in the preparation of this paper included eleven cases from the surgical laboratory of the Toronto General Hospital, six cases from the laboratories of the Department of Health, Ontario, and two cases from the Hospital for Sick Children, one of which was an example of Gaucher's disease. In all but the latter case, the xanthoma nodules were removed by surgical procedures since 1929, making a total of eighteen specimens.

Xanthoma has been found in nearly every tissue of the body.<sup>1</sup> Probably the most frequently seen type of xanthoma is the so-called xanthelasma palpebrarum. The disease appears as flattened yellowish plaques on the upper eyelids of older people. The next most common type is the xanthoma of the tendon sheaths. Certain conditions, such as diseases of the liver with icterus, nephritis and diabetes, are frequently associated with the formation of xanthoma. Specialized types of xanthoma forming definite clinical syndromes, such as Schüller-Christian's disease, Gaucher's disease and Niemann-Pick disease, are not common. The familial incidence of xanthoma has been observed and recorded by many.<sup>2</sup>

Xanthoma usually presents a typical appearance in the gross. It is remarkable that as a general rule one may recognize xanthoma and similar processes by their peculiar color. The typical xanthelasma seen on the upper eyelids of persons past middle life presents a yellowish-orange color, sometimes with a grayish tint. This color may or may not be seen in other types of xanthoma, as in xanthoma diabeticorum,

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which usually has a zone of redness about the borders of the nodule. Or again, in xanthoma tuberosum and xanthoma multiplex, the color may not be obvious from the surface. If, however, one incises such a nodule, the typical egg-yolk color may be seen. In this regard it is noteworthy that atheroma of the arteries is identical in color to xanthoma. The color here may be modified considerably by the occurrence of atheroma in a large plaque of chronic nodular endarteritis, in which case the yellowish color may be faintly seen through the layer of the pearly white endarteritis. Furthermore, in locations where the tissues have been involved in chronic suppurative reaction, masses presenting this typical color may be seen; for example, chronic empyema, chronic abscess of the brain and chronic suppurative salpingitis. Smith<sup>3</sup> claimed that this color may be due to a colored lipid, an endogenous melanotic pigment, blood pigment or a combination of these. Garret<sup>4</sup> ascribed the color to blood pigment. Miller<sup>5</sup> claimed that carotene and xanthophyll, belonging to the group of carotinoids, when combined with cholesterol esters, which themselves are colorless, give rise to this color.

In preparing microscopic sections for study, the following stains were used after embedding in paraffin: hematoxylin and eosin, Mallory's connective tissue stain, phosphotungstic acid-hematoxylin and Masson's stain. A special study was made of frozen sections which were stained with sudan III and Nile blue, and further differentiation was obtained by the use of the polarizing microscope.

Pollitzer and Wile<sup>6</sup> grouped their microscopic observations into three stages: the smallest and youngest lesions, the stage of proliferation and the stage of retrogression. Their findings were in general substantiated by the present series. In the earliest lesions a few foam cells may be seen in intimate relationship to the adventitia of small blood vessels. These cells vary from 10 to 25 microns in diameter. They are very pale in paraffin sections. A fine thin cell membrane encloses a cytoplasm which consists of very delicate strands forming tiny, round, oval and slitlike spaces. The cytoplasm is slightly basophilic in reaction. The nucleus is usually centrally placed and is large and generally pale-staining; frequently a nucleolus can be seen. In sections stained with sudan III, tiny red globules can be seen to fill most of the cell. Sometimes these globules are larger and fewer. When Nile blue is used, these globules stain faintly pink, deep blue or an intermediate mauve to purple. Under the polarizing microscope a small amount of doubly refractile substance can be seen. These fat-staining reactions indicate a mixture of lipid substances, and since the nucleus shows no

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5. Miller, quoted by Rowland, R. S.: Ann. Int. Med. 2:1277, 1929.

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qualities demonstrative of a degenerative process, it is assumed that this lipoid material has been phagocytosed by the aforementioned cells.

In later lesions, the number of foam cells is greatly increased. Individually, they are larger, measuring up to 40 microns in diameter. The lipoid globules become smaller. There is a more definite appearance to the cytoplasm. It appears as a fine network of a reticulated character, slightly basophilic in reaction. The nucleus is sometimes swollen, and the chromatin network is coarse and deeply stained. Other nuclei are pale and dotted with finely granular chromatin, and usually an eccentric nucleolus is present. There is considerable fibrous connective tissue round about masses of the foam cells. Some of the individual foam cells appear compressed and elongated by pressure from the surrounding fibrous tissue cells. Occasionally, with fat stains, fibroblasts may be seen to contain a small amount of lipoid material, none of which, however, is doubly refractile to polarized light. In a large clump of foam cells, the more central cells may become polygonal. Occasionally a large cell may be seen with two or three nuclei and foamy cytoplasm, evidently a stage in the formation of a Touton giant cell. This picture is to be differentiated from the foreign body giant cell, which infrequently may be seen enclosing a long narrow slit, representing the position of a cholesterol crystal which has been dissolved in the preparation.

At a later stage there is a still greater increase in the connective tissue element, which at times closely resembles granulation tissue. The foam cells are of a more uniform size. Many cells having the appearance of giant cells may, on closer examination, be seen to be true giant cells, or, on the other hand, a thinning of the cell membrane may closely simulate this condition. On examining this stage with Nicol prisms, the cholesterol content of the cells appears to have definitely increased, although the reactions to the fat stains remain as before.

In none of the cases examined was the xanthoma seen to extend into the epidermis or below the deep fascial layers. In every case the lesion was limited to the subcutaneous tissues, which in these areas were more or less thickened.

In my observations on the fatty character of these lesions, Nile blue was used to distinguish neutral fat from fatty acid, the former staining red and the latter blue. Thus the lipoid constituent of xanthoma was found to be composed of cholesterol and its esters, neutral fats and fatty acids. Probably the most important and constant of these is cholesterol and its esters. The relation between these lesions and a possible disturbance of cholesterol metabolism has thus been the object of great attention.

Many authors have observed and reported cases of xanthoma associated with hypercholesteremia (Ingram,<sup>7</sup> Hoessli<sup>8</sup> and others). A few have reported the blood cholesterol as normal or even subnormal.<sup>9</sup> In this regard Bloch<sup>10</sup> pointed out that low serum cholesterol was the exception rather than the rule. He showed that the lipid system of the blood consisted of the insoluble substances cholesterol, phosphatides, neutral fats and fatty acids, which were present in a finely dispersed stable emulsion, and soaps, which were soluble. Proportions of the substances, if changed sufficiently from the normal, resulted in a disturbance of the stable emulsion, the particles becoming larger and coarser and, granted favorable conditions, being precipitated to form xanthoma. Thus, Bloch showed that determination of only one constituent (viz., cholesterol) was not conclusive in establishing the presence or absence of a disturbance of lipid proportions.

Disturbance of lipid metabolism and especially hypercholesteremia can thus be seen to play an important etiologic rôle in the production of xanthoma.

Such conditions as jaundice<sup>11</sup> and diabetes (Finney, Montgomery and New,<sup>1</sup> Major<sup>12</sup> and others) are frequently precursors to the formation of xanthoma. Numerous other authors (Engman and Weiss,<sup>13</sup> Mook and Weiss<sup>14</sup> and others) have observed the disappearance of xanthoma in these cases with suitable diet or treatment with insulin.

Trauma as an etiologic factor has been advanced by several writers. The frequent occurrence of xanthoma of the tendon sheaths possibly bears some relation to friction as a localizing factor. Chauffard<sup>15</sup> presented a case in which xanthoma developed at the site of hypodermic injections of sodium cacodylate, the patient having a preexisting hypercholesteremia. Ochs<sup>16</sup> described a case in which xanthoma developed at the sites of injection during treatment for syphilis and in which a series of lesions developed around the vaccination scar. Many experimenters have produced a state of hypercholesteremia in animals, espe-

7. Ingram, J. T.: *Brit. J. Dermat.* **39**:335, 1927.

8. Hoessli, H.: *Beitr. z. klin. Chir.* **95**:185 and 198, 1914.

9. Rosenthal, F., and Braurnisch, R.: *Ztschr. f. klin. Med.* **92**:429, 1921.  
Rosenbloom, J.: *Arch. Int. Med.* **12**:395, 1913.

10. Bloch, B.: *Brit. J. Dermat.* **43**:61, 1931.

11. Hutchinson, J.; Sangster, A., and Crocker, H. R.: *Tr. Path. Soc. London* **33**:376, 1882.

12. Major, R. H.: *Bull. Johns Hopkins Hosp.* **35**:27, 1924.

13. Engman, M. F., and Weiss, R. S.: *Arch. Dermat. & Syph.* **8**:625, 1923.

14. Mook, W. H., and Weiss, R. S.: *Arch. Dermat. & Syph.* **8**:19, 1923.

15. Chauffard, quoted by McWhorter, J. E., and Weeks, C.: *Surg., Gynec. & Obst.* **40**:199, 1925.

16. Ochs, B.: *Arch. Dermat. & Syph.* **22**:922, 1930.

cially the rabbit, by feeding them a diet high in cholesterol. Anitschow<sup>17</sup> produced subcutaneous sterile abscesses in rabbits in this state and produced typical xanthoma. Weidman<sup>18</sup> produced a similar condition in dogs. Chuma<sup>19</sup> produced xanthosis and xanthoma in rabbits under similar circumstances. Anitschow also produced localized xanthoma in hypercholesteremic rabbits by repeated trauma to the skin over the tendo achillis and by freezing the skin with carbon dioxide.

From the foregoing remarks it would appear that disturbance of cholesterol metabolism is of definite etiologic significance, and that trauma may be a factor in the localization of the xanthomatous process.

In a similar manner, the so-called atheroma may be experimentally produced by feeding animals a diet high in cholesterol.<sup>20</sup> The first lesions produced by experimental methods are the fine superficial fatty streaks.<sup>20d</sup> If carried for a sufficient time, atheroma may be produced.<sup>21</sup> Klotz found that the name "atheroma" was given by Haller in 1755 to "callosities and yellow spots projecting into the arterial lumen from which a soft pultaceous material could be expressed."

In the gross the appearance of atheroma simulates that of xanthoma remarkably. The peculiar yellowish-orange color is an outstanding feature. Atheroma appears as localized, flattened nodules or plaques slightly raised above the remainder of the intimal surface. It has, however, two features which are not seen in xanthoma: (1) On incising the intimal covering, a grumous, slimy, yellowish material may be squeezed out; and (2) there is a tendency toward calcification with advancing years.

On microscopic examination a significant finding, which sometimes is very marked, is the presence of foam cells. These have an appearance identical to that of the foam cells discussed previously as xanthoma cells. The features of the large pale cell with very fine vacuoles, giving to the cytoplasm a finely reticular structure in sections prepared with alcohol, may be seen. The size of these cells varies from 10 to 35 microns. The nuclei usually stain well but show no evidences of degeneration. Moreover, on employing fat stains, the material in these cells may be seen to consist of cholesterol esters, fatty acids and neutral fats.

Even in the earliest lesions of fatty nature in the intima, that is, superficial fatty streaks, foam cells may be seen.<sup>20d</sup> These cells are large

17. Anitschow, N.: (a) *München. med. Wchnschr.* **60**:2555, 1913; (b) *Beitr. z. path. Anat. u. z. allg. Path.* **57**:201, 1914.

18. Weidman, F. D.: *Arch. Dermat. & Syph.* **15**:659, 1927.

19. Chuma, M.: *Virchows Arch. f. path. Anat.* **242**:275, 1923.

20. (a) Klotz, Oskar: *Brit. M. J.* **11**:1767, 1906; (b) *J. M. Research* **33**:157, 1915; (c) *Centralbl. f. allg. Path. u. path. Anat.* **19**:535, 1908; (d) Duff, G. L.: *Experimental Cholesterol Arteriosclerosis*, to be published.

21. Klotz, Oskar: *J. M. Research* **32**:27, 1915.

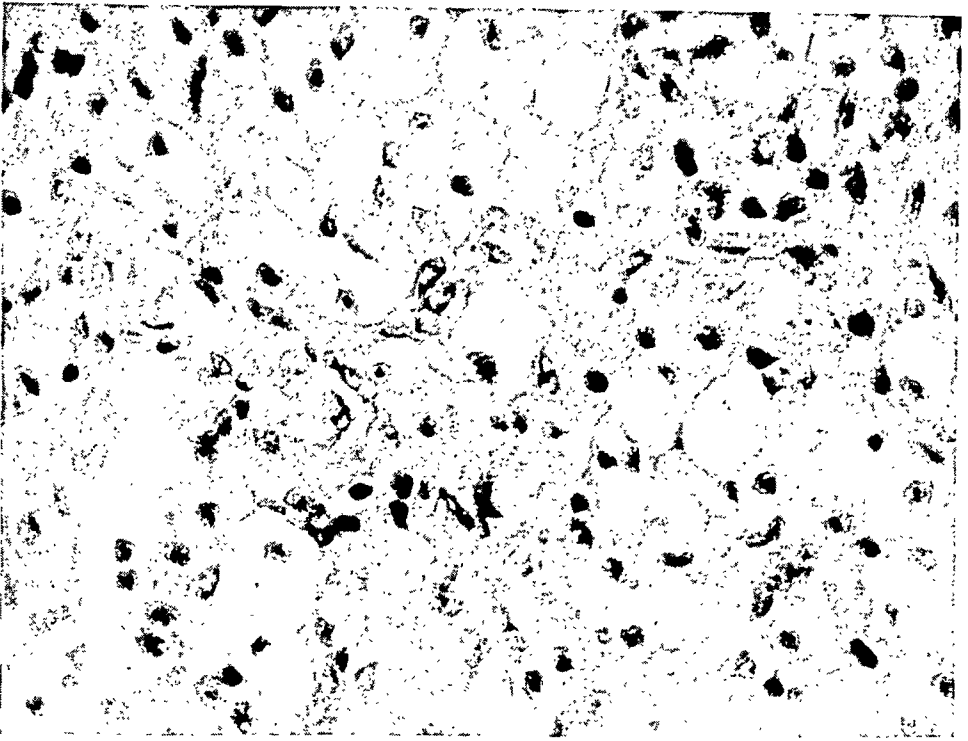


Fig. 1.—Chronic pelvic suppurative reaction showing foam cells. Hematoxylin and eosin stain;  $\times 400$  (A-81-33).

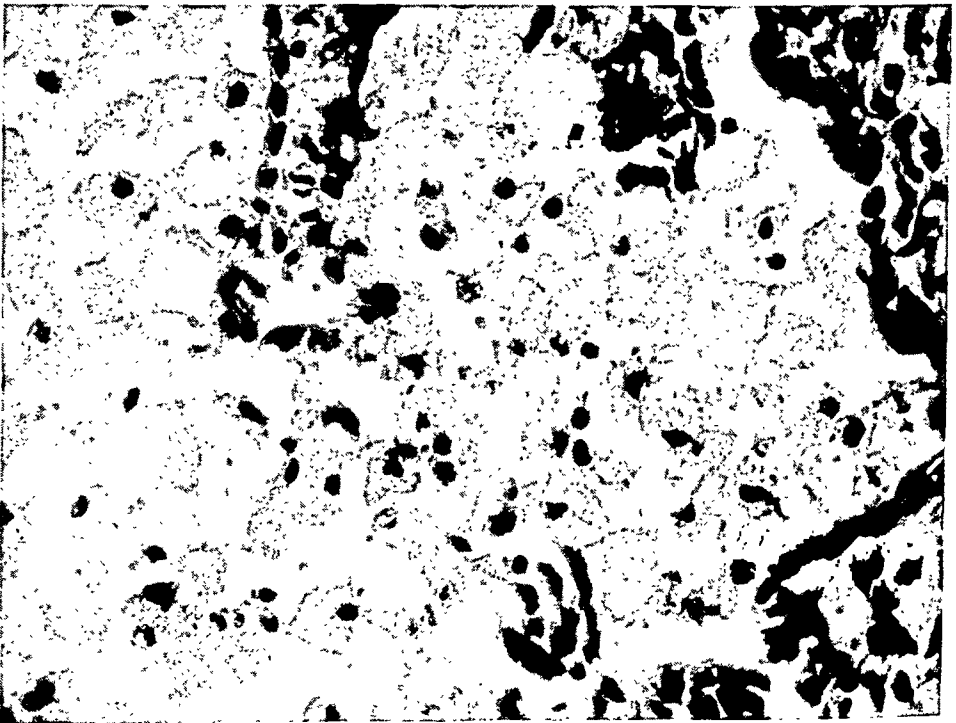


Fig. 2.—Section of lung (inhaled peanut with sequelae) showing foam cells in the alveoli. Hematoxylin and eosin stain;  $\times 400$  (A-387-32).

pale cells loaded with fatty substance, but not usually having the finely reticular character, as mentioned. It is also significant that cholesterol and its esters can rarely be demonstrated in these cells with the polarizing microscope. Extracellular fat and fibroblasts containing fat are found. In more advanced lesions of the superficial fatty streak variety, true foamy cells are frequently observed, and anisotropic fatty substances may be demonstrated.

In one case sections through the aorta revealed a remarkable picture. The intima was greatly thickened and greatly vascularized. Many capillaries were present, and surrounding these capillaries, in intimate relation to them, were large numbers of typical foam cells. The material studied was obtained during an autopsy (fig. 5) in a case in which thorotrast had been used clinically for diagnostic purposes. Thorotrast is a suspension of finely particulate thorium dioxide, and has been used recently as a method of outlining the reticulo-endothelial system (Irwin<sup>22</sup>), the cells of this system displaying an active phagocytic action toward particulate matter in the blood stream. It is significant and, I believe, an important finding that after a careful study thorotrast was found in small quantity within foam cells making up atheroma of the aorta. A few small, highly refractile particles were seen within typical foam cells surrounding the capillaries in the thickened intimal atheromatous plaque. The particles of thorium dioxide were usually to be seen at the periphery of the finely reticular cytoplasm and were demonstrated best in sections stained with hematoxylin and eosin and Masson's stain. The significance of this finding will be discussed later.

Foam cells may be seen in other conditions, and numerous authors have called attention to this fact (Haagensen,<sup>23</sup> Aschoff,<sup>24</sup> Harbitz<sup>25</sup> and Bloch<sup>10</sup>). Thus, in routine work several instances have been observed in a relatively short period. One example was found in connection with a chronic pelvic inflammation in which numerous small yellowish-orange nodules were found; on section of the nodules, fat stains and paraffin sections revealed the typical foam cells described, with a lipoid content similar to the xanthoma cell. Other examples were chronic abscess of the brain, chronic empyema and strawberry gallbladder. Another interesting case was that of a patient who died as a result of pneumonia following aspiration of a peanut several months previously. In this case sections of the lung presented the typical egg-yolk color in the gross; on microscopic examination, in many areas the alveoli were plugged with typical foam cells. The fat stains here again disclosed the lipoid content to be of a similar nature. In addition to those given,

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22. Irwin, D. A.: *Canad. M. A. J.* **27**:130, 1932.

23. Haagensen, C. D.: *Am. J. Cancer* **16**:1077, 1932.

24. Aschoff, quoted by Brown, T. R., and Howard, J. T.: *Internat. Clin.* **4**: 106, 1931; quoted by Hoessli.<sup>8</sup>

25. Harbitz, F.: *Arch. Path.* **4**:507, 1927.

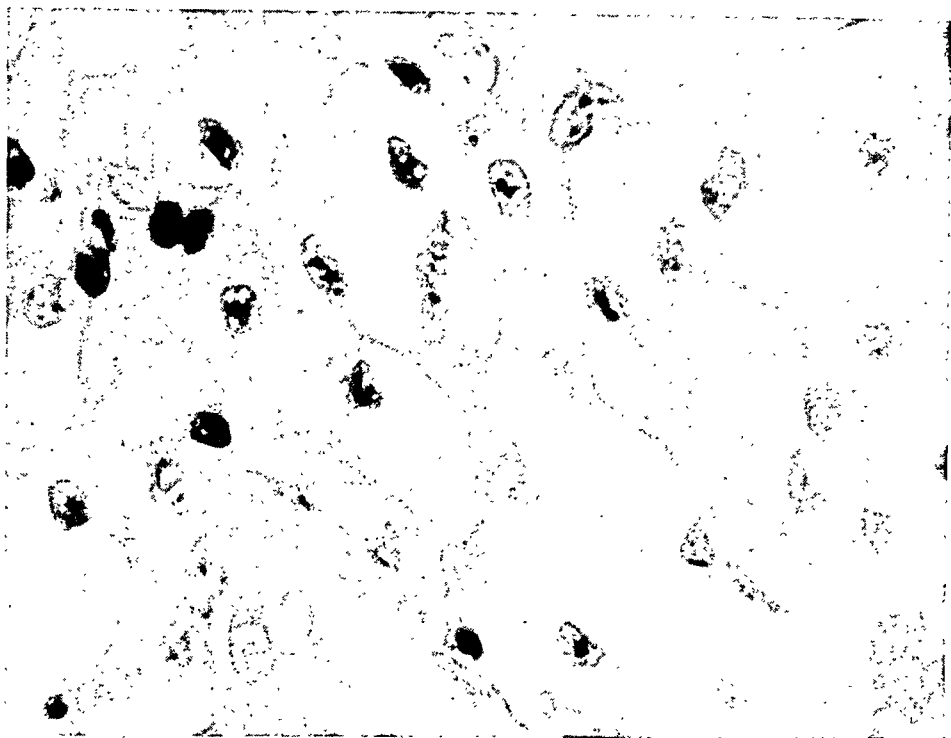


Fig. 3.—Xanthelasma palpebrarum, showing Touton giant cell and many foam cells. Hematoxylin and eosin stain;  $\times 800$  (U.S.C.-S-642-32).

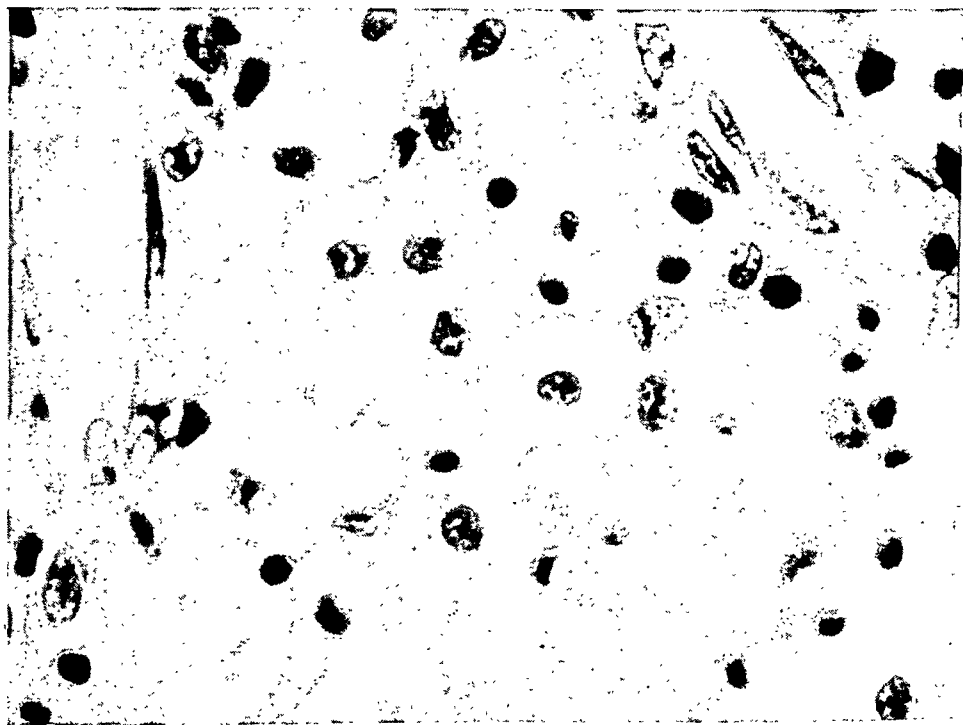


Fig. 4.—Xanthoma of the tendon sheath showing relation of the foam cells to the capillaries. Hematoxylin and eosin stain;  $\times 800$  (Dr. Bates T-888-31).

Stewart<sup>26</sup> listed the following conditions: cholesteatoma of the choroid plexus, myelin kidney, myeloid tumor of the tendon sheaths (endothelioma), subacute inflammation of the adipose tissue, subacute and chronic salpingitis, subacute and chronic abscesses, retention lesions of the breast, cerebral lesions in which there is degeneration of the myelin, dermoid cysts, mycosis fungoides, certain lesions of the thyroid gland and tumors showing degeneration. It will be seen that xanthoma and atheroma are found in cases with a probable derangement of cholesterol metabolism and are, therefore, generalized conditions. On the other hand, the remaining lesions are associated with a local degenerative

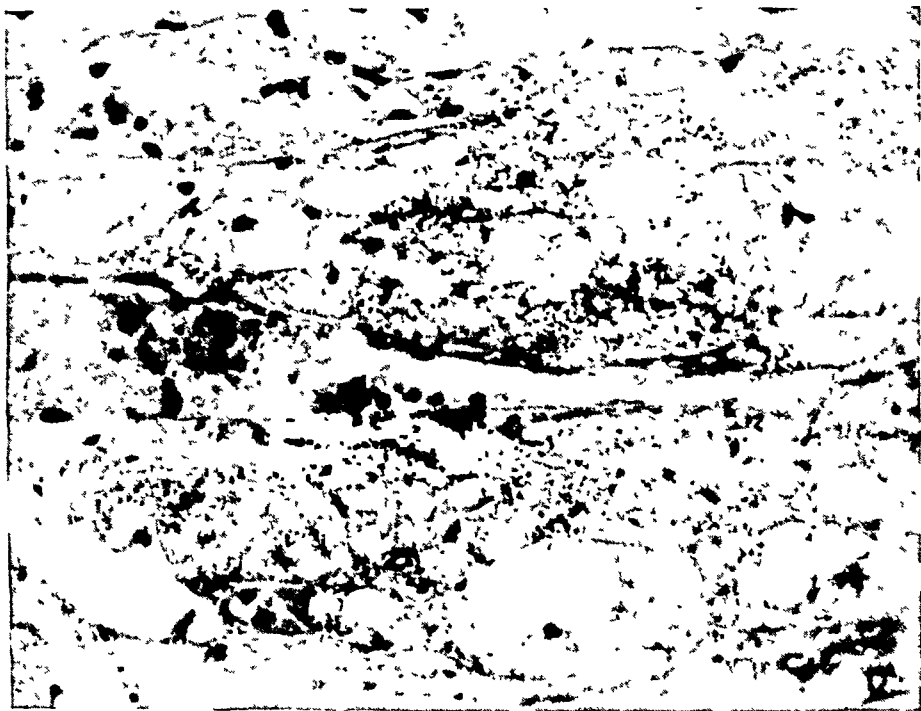


Fig. 5.—Aorta, showing vascularization of the intima with atheroma and foam cells containing thorotrast. Hematoxylin and eosin stain;  $\times 400$  (A-72-33).

process in which there is liberation of lipid locally. Weber<sup>27</sup> added another case, one of arcus senilis, and in relation to all of these conditions, he spoke of a cholesterol diathesis.

Thus, it may be seen that certain types of lesions, characterized by a typical gross picture and consisting mainly of foam cells as seen under the microscope, are intimately linked together. On the one hand, there may be as an etiologic factor hypercholesteremia, or, again, an alteration in the lipid constituents so that cholesterol is precipitated out. And, on the other hand, in certain other conditions in relation to chronic sup-

26. Stewart, M. J.: *Brit. M. J.* 2:893, 1924.

27. Weber, F. P.: *Brit. J. Dermat.* 36:335, 1924.



puration, a localized precipitation or infiltration of cholesterol esters occurs.<sup>28</sup> In all of these conditions foam cells may appear. Moreover, it is also noteworthy that in normal tissues not belonging to the reticulo-endothelial system, such as the lutein cells of the ovary, the cortex of the suprarenal glands and the sebaceous glands, cells with foamy cytoplasm are seen; this is related to a local increase in cholesterol in these tissues. I shall leave for the present consideration of Gaucher's disease and Niemann-Pick disease, since these conditions have been found to be associated with lipoids other than cholesterol.

When cholesterol is present in large amounts or when lipid ratios are altered so that the cholesterol exists as finely particulate matter in colloidal suspension, it is phagocytosed by the cells of the reticulo-endothelial system. When these cells have performed this function, they gradually assume the form of the foam cell. What proof is there that the xanthoma cells are of the reticulo-endothelial system? By their very function of phagocytosis of particulate matter and colloidal material, they proclaim themselves as such. Moreover, Anitschow,<sup>17a</sup> in his experimentally produced xanthoma, found that the cells which phagocytosed the lipid material picked up vital dyes. In a similar manner, I have demonstrated thorotrast, which is comparable to a vital dye in this regard, within foam cells in atheroma of the aorta. Thus I have shown not only that the foam cell of atheroma is probably identical in function to the xanthoma cell, but also that both probably arise from the same system of cells, that is, the reticulo-endothelial system. That lipoids other than cholesterol may act in this manner is demonstrated in the more rare diseases, Gaucher's disease and Niemann-Pick disease, the former being associated with a lipoprotein and the latter with a phosphatide.<sup>29</sup>

#### CONCLUSIONS

A series of eighteen cases of xanthoma, in which the nodules were removed surgically, was studied from a standpoint of the origin and nature of the xanthoma cell.

A study of fatty lesions of the arteries was made in relation to a fundamental similarity of atheroma and xanthoma.

It is believed that the xanthoma cell and the foam cell of atheroma, of chronic suppurative reactions and of certain systemic diseases are of identical nature.

Furthermore, I believe that the origin of both of these cells is from the reticulo-endothelial system, and that the foam cell is an evidence of specific reaction of the cells of the reticulo-endothelial system to certain lipoids, especially cholesterol and its esters, when conditions favorable for their deposition in tissues are present.

28. Landois, F., and Reid, M.: *Beitr. z. klin. Chir.* **95**:56, 1914.

29. Sosman, M. C.: *Am. J. Roentgenol.* **23**:581, 1930.

# MULTIPLE NECROSES OF THE SPLEEN (FLECKED SPLEEN OF FEITIS)

WITH SPECIAL REFERENCE TO THE ASSOCIATED RENAL LESIONS

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In 1921, Feitis<sup>1</sup> reported a thorough study of two cases of multiple anemic necroses of the spleen which differed in both gross and microscopic structure from the forms described previously. The rarity of this condition is apparent, as only twenty-one cases have been described in the literature since Feitis' publication. These are briefly outlined in table 1.

The appearance of the spleen is characteristic. The cut surface shows many well defined, light yellow to gray-white areas which are firm and vary in diameter from 2 or 3 mm. to 2 cm. Frequently they are crowded closely to the peripheral portion of the spleen and are absent in the center, although in some cases the entire organ is involved. These areas are very irregular and of bizarre shape and frequently show jagged outlines. Many of the larger foci are connected by narrow bands of necrotic tissue, giving the surface a mosaic structure. These light-colored areas stand out conspicuously against the dark red-brown pulp. A distinct peripheral zone of hyperemia may be present around many of the necrotic areas. In some areas this is lacking. The organ occasionally shows marked atrophy. In most cases the spleen is of normal size, and in a few cases its weight is increased (Nicod;<sup>2</sup> Meuret,<sup>3</sup> case 2; Adolphs,<sup>4</sup> case 2).

On microscopic examination Feitis<sup>1</sup> distinguished two types of necrosis: (1) typical necrosis and (2) atypical necrosis. The typical necrosis conforms somewhat to the structure of an infarct, in that three zones are distinguishable: a necrotic central area composed of a structureless, dense mass of nuclear débris and necrotic parenchyma; a paler intermediate zone, or *Auslaugungszone*, containing necrotic parenchyma infiltrated with a few leukocytes, and a peripheral hemorrhagic zone which merges with the non-necrotic parenchyma. These areas are most numer-

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1. Feitis, H.: Beitr. z. path. Anat. u. z. allg. Path. **68**:297, 1921
2. Nicod, J. L.: Ann. d'anat. path. **7**:67, 1930.
3. Meuret, W.: Beitr. z. path. Anat. u. z. allg. Path. **73**:535, 1924.
4. Adolphs, E.: Frankfurt. Ztschr. f. Path. **41**:433, 1931.

TABLE 1.—Cases of Flecked Spleen Found in the Literature

Author	Sex; Age, Years	Size of Heart	Blood Pressure		Cause of Death	Renal Changes	Appearance of Spleen
			Sys- tolic	Dias- tolic			
Feltis, 1921 Case 1	39	870 Gm.	240	...	Uremia	"Genuine contracted kidney" (arterio-sclerosis)	Weight, 205 Gm.; discrete and confluent irregular areas of anemic necrosis; degeneration of small and medium-sized arteries with occlusion; arteriosclerosis and thrombosis of larger arteries
Feltis, 1921 Case 2	Male 60	Marked hypertrophy of left ventricle	...	...	Uremia	"Horseshoe kidney with granular atrophy"	Atrophy; granular surface; large areas of necrosis beneath capsule; microscopic changes similar to those in case 1
Meuret, 1924 Case 1	31	Marked hypertrophy	...	...	Uremia	"Nephrosclerosis arterio-sclerotica"	Infarct-like areas replacing one third of spleen; obliteration of small arteries; secondary thrombosis of larger arteries
Meuret, 1924 Case 2	46	Hypertrophy and dilatation	200	...	Cerebral hemorrhage	"Hydronephrosis left; malignant nephrosclerosis right;" infarcts	Enlarged, firm and maculated; hyperplastic intimal changes in larger arteries; necrosis and occlusion of small arteries
Lubarsch, 1926 Case 1	..	Marked hypertrophy of left ventricle	...	...	Cardiac failure	"Contracted kidneys and uric acid deposits"	Weight, 215 Gm.; numerous areas of anemic necrosis; necrotic changes in small arteries; thrombosis and inflammation of larger arteries
Lubarsch, 1926 Case 2	Female 44	Marked hypertrophy of left ventricle	...	...	Cardiac failure	"Marked granular atrophy of both kidneys"	Small and indurated; fresh areas of anemic necrosis; arterial changes similar to those in case 1
Lubarsch, 1926 Case 3	Male 52	705 Gm.; hypertrophy of left ventricle	...	...	Cardiac failure	"Granular atrophy"	Weight, 130 Gm.; small areas of necrosis connected by strands; arterial changes as in case 1
Lubarsch, 1926 Case 4	Male 53	.....	...	...	Chronic uremia	"Atrophy of both kidneys"	Almost complete anemic necrosis due in part to arterial emboli and in part to arterial changes similar to those in case 1
Hosoi, 1928	Female 45	Clinically enlarged	230	140	Cerebral hemorrhage	"Chronic vascular nephritis"	Weight, 110 Gm.; irregular nodular maplike yellow areas; arterioles obliterated; fresh thrombosis of larger arteries

Kabakakis, 1930	Male 38	Marked hypertrophy of left ventricle	210	150	Uremia	Marked atrophy; chronic glomerulonephritis; arteriosclerosis	Large; many discrete and confluent areas of necrosis; necrosis and occlusion of small and medium-sized arteries
Nicol, 1930	Female 48	500 Gm.; hypertrophy of left ventricle	250	180	Uremia	Moderate atrophy; "chronic glomerulonephritis; arteriosclerosis"	Weight, 250 Gm.; firm, small yellow areas beneath capsule; proliferative intimal changes and hyalinization of small arteries
Klemperer and Otani, 1931	Female 46	Hypertrophy of both ventricles	210	135	Uremia; cardiac failure	Atrophy; "malignant nephrosclerosis"	Firm and nodular; mosaic in appearance due to multiple areas of necrosis
Adolphs, 1931 Case 1	Female 33	Hypertrophy	250	...	Cerebral hemorrhage	"Chronic glomerulonephritis with arteriolonecrosis"	Surface smooth; irregular pointed areas of necrosis beneath capsule and in center; necrotic changes in small and medium-sized arteries
Adolphs, 1931 Case 2	Male 57	Hypertrophy	...	...	Hemorrhage from ulcer	"Chronic glomerulonephritis," arteriolonecrosis and endarteritis	Enlarged; many irregular yellow areas of necrosis, more numerous in center; vascular changes similar to those in case 1
Adolphs, 1931 Case 3	Female 11	Dilatation	...	...	Uremia	"Early acute glomerulonephritis;" necrosis of vasa afferentia	Gray-yellow areas of necrosis in center; necrosis and occlusion of small arteries
Spler, 1931	Female 51	540 Gm.	...	...	Apoplexy	"Arteriolosclerosis"	Weight, 92 Gm.; multiple areas of necrosis beneath capsule; necrosis and occlusion of lumen of small arteries
Rake Case 1	Female 44	Hypertrophy of left ventricle	...	...	Uremia	"Arteriolosclerosis and arteriolonecrosis"	Weight, 140 Gm.; multiple infarct-like areas; occlusion of small arteries due to necrosis and thrombosis
Rake Case 2	Female 52	.....	...	...	Uremia	"Malignant nephrosclerosis"	Changes similar to those in case 1, except that degenerative arterial changes are more marked
Göpel, 1925	Female 35	.....	...	...	Eclampsia	"Multiple necrosis"	Weight, 330 Gm.; multiple yellow, confluent areas of necrosis throughout spleen; fibrinous thrombi occluding small arteries
Laufer, 1933 Case 1	Male 58	.....	...	...	.....	"Subacute-chronic glomerulonephritis"	Size, 13 by 7.5 by 5 cm.; multiple irregular areas of necrosis; acute endarteritis and periarthritis
Laufer, 1933 Case 2	Male 37	Hypertrophy of left ventricle	...	...	Uremia	"Chronic glomerulonephritis"	Size, 15 by 8.5 by 4.5 cm.; appearance of spleen and vascular changes similar to case 1

ous near the capsule. The areas of atypical necrosis are smaller and less frequently seen than the typical forms. They do not possess the three zones just described and vary considerably in structure. Large areas of necrosis are formed by the fusion of a number of smaller areas. There is no relation between the lymphoid follicles, trabeculae and pulp and the necrotic areas.

The vascular changes are pronounced, being most marked in the small and medium-sized central arteries of the follicles. In the majority of cases reported, these changes consist of necrosis of the wall which begins in the intima and extends into the media, causing confluence of these two layers. The walls are converted into a hyaline coagulated mass containing scattered nuclear fragments. In many of these vessels the endothelium is destroyed and the lumen is filled with a hyaline substance which merges with the necrotic walls of the vessel. Marked hyperplastic elastic intimal thickening and regenerative connective tissue proliferation of the intima are present in the larger vessels of the trabeculae. Occasionally, these vessels contain partially organized thrombi. With the exception of one case (Lubarsch,<sup>5</sup> case 4) embolic phenomena have been absent. The veins are not affected primarily.

Geipel<sup>6</sup> reported a case of puerperal eclampsia in which splenic changes similar to those described by Feitis<sup>1</sup> were noted. These changes were associated with multiple necroses of the cortex of the kidneys. The vascular changes in this case differed from those described in the other cases cited. The small arteries of the red pulp, the central arteries of the lymphoid follicles and a few of the small trabecular arteries were partially or completely occluded by fibrin thrombi. The peculiar necrosis of the arterial walls seen in other cases of flecked spleen was lacking in Geipel's case. The larger arteries were not affected. Mathias, in a discussion of Geipel's<sup>6</sup> paper, stated that he had seen similar changes in the spleen of an eclamptic patient showing the typical changes in the liver described by Schmorl. This case is not included in table 1, since no description is given. Mathias expressed the opinion that the necrosis was due to angiospasm followed by endothelial injury and thrombosis. Lubarsch<sup>5</sup> maintained that in these cases the thrombosis and subsequent necrosis are due to a toxic factor, and he accordingly designated this condition as the "toxic-thrombotic" form of flecked spleen, in contradistinction to the type described by Feitis,<sup>1</sup> Meuret<sup>3</sup> and others, which

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5. Lubarsch, O.: *Pathologische Anatomie der Milz*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histology*, Berlin, Julius Springer, 1927, vol. 1.

6. Geipel: *Centralbl. f. allg. Path. u. path. Anat.* **35**:8, 1924; *Arch. f. Gynäk.* **124**:231, 1925.

he classed as "arteriosclerotic autotoxic" flecked spleen. Hosoi<sup>7</sup> adopted a similar classification but incorrectly included in the toxic-thrombotic group a case of pernicious anemia described by Lubarsch<sup>5</sup> in which multiple necroses of the spleen occurred following blood transfusion. In this case thrombosis was completely lacking, and for this reason Lubarsch hesitated to include it in the toxic-thrombotic group or to classify it as a form of flecked spleen. As a toxic cause of thrombosis of the small arteries in eclampsia and the pathogenesis of arteriolonecrosis are still disputed, a classification of flecked spleen based on etiologic pathologic information is not justifiable. It would be less confusing to regard the type of lesion described by Geipel as a thrombotic form of flecked spleen, as distinguished from the arteriosclerotic form described by Feitis and others.

The existence of a third, or arteritic, type of flecked spleen is indicated by the recent case reports of Laufer.<sup>8</sup> The lesion is inflammatory, involving the small and medium-sized arteries of the spleen. Laufer found marked leukocytic infiltration of the adventitia and media, frequently with necrosis and swelling of the media and connective tissue proliferation of the intima. Laufer classified the lesion as periarteritis nodosa. The vascular changes, however, do not conform entirely to the classic picture of periarteritis nodosa described by Kussmaul and Maier,<sup>9</sup> since the arteries of visible caliber are not involved, aneurysmal dilatations are not described and thrombosis is lacking. Moreover, the areas of anemic necrosis of the spleen do not resemble the infarct-like lesions found in classic cases of periarteritis nodosa. The type of vascular lesion described by Laufer is similar to that seen occasionally in cases of subacute glomerulonephritis (Klemperer and Otani<sup>10</sup>) and in cases of malignant hypertension (Fahr<sup>11</sup>). Klemperer designated these lesions as necrotizing arteritis, endarteritis and periarteritis, as distinguished from the purely degenerative vascular type, or arteriolonecrosis, seen in most cases of malignant hypertension.

Enzer,<sup>12</sup> in 1926, described a case of multiple necroses of the malpighian bodies in a case of pernicious anemia. Death was due to bronchopneumonia. Although Enzer included the case as a typical

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7. Hosoi, K.: *Arch. Path.* **6**:26, 1928.

8. Laufer, S.: *Centralbl. f. allg. Path. u. path. Anat.* **58**:113, 1933.

9. Kussmaul and Maier, quoted by Jores, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol. 2.

10. Klemperer, P., and Otani, S.: *Arch. Path.* **11**:60, 1931.

11. Fahr, T.: *Pathologische Anatomie des Morbus Brightii*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1925, vol. 6, pt. 1.

12. Enzer, N.: *Am. J. Path.* **2**:511, 1926.

example of flecked spleen, differing only in etiology, there seems to be meager evidence for this assumption. The large, irregular, connected areas of necrosis, easily visible to the naked eye, found in all the cases reported in table 1, were not present in Enzer's case, the necrotic areas being extremely small and barely visible to the naked eye. There were no evidences of vascular disease, such as obliteration of the lumens or the formation of thrombi. There is little ground for Enzer's assumption that flecked spleen of Feitis is characterized by necrosis of the malpighian bodies. No description of this is given in the papers of Feitis,<sup>1</sup> Lubarsch,<sup>5</sup> Meuret,<sup>3</sup> Adolphs,<sup>4</sup> and others. Feitis stated that no relationship could be found between the areas of necrosis and the malpighian bodies. In many of the cases, the lymphoid tissue is very scant and consists of narrow cylindric sheaths about the central arteries. Feitis<sup>1</sup> was unable to find changes other than fibrosis and occasionally hyalinization of the lymphoid tissue. When the follicles are included in the areas of necrosis, their cellular structures appear much better preserved than other structures about them.

The splenic changes described by Enzer in all probability belong to the group of infectious toxic necrosis not infrequently seen in various infectious diseases, such as typhus, acute and subacute endocarditis, scarlet fever and diphtheria. It resembles particularly the changes sometimes seen in scarlet fever and diphtheria, in which the necrosis is limited to the malpighian corpuscles.

Wilton's<sup>13</sup> case and case 3 of Lauber<sup>8</sup> are somewhat similar to the one reported by Enzer. In Wilton's case, the spleen was considerably enlarged and beset with numerous small white areas and a few large irregular foci resembling somewhat the picture seen in tuberculous caseative necrosis and abscess formation involving the malpighian bodies. The central arteries of the follicles in the region of the necrotic areas were thrombosed. The thrombosis was secondary to the perivascular necrosis. Wilton believed that there was marked similarity between this case and that described by Feitis. Lubarsch,<sup>5</sup> however, was inclined to the view that the two conditions are different. It is more likely that, as in Enzer's case, the condition described by Wilton is a type of necrosis and multiple abscess formation of the malpighian bodies encountered in various infectious diseases. Lauder's case 3 similarly was associated with streptococcic septicemia in which multiple abscesses, infarct-like lesions and acute arteritis with thrombosis were noted.

#### CHANGES IN THE KIDNEYS

The interpretation of the renal lesions in the cases of flecked spleen described in the literature offers considerable difficulty, especially in those

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13. Wilton, A.: Frankfurt. Ztschr. f. Path. **31**:110, 1925.

cases in which a detailed microscopic picture is not given. The renal condition, with few exceptions, is associated with essential hypertension and uremia. The diagnosis is extremely varied, as noted in table 1. Such terms as arteriolosclerosis, malignant hypertension, vascular nephritis, nephrosclerosis arteriosclerotica, granular atrophy and chronic glomerulonephritis with arteriolonecrosis are used to indicate identical or closely related conditions. This disagreement in terminology is readily understood since there is still little agreement, especially among pathologists, as to the exact nature of the renal disease in essential hypertension.

Volhard and Fahr,<sup>14</sup> in 1914, stated that hypertension with renal insufficiency differs in its pathogenesis from the form without renal insufficiency, or benign hypertension. The former, termed *Kombinationsform*, was thought to be due to a combination of arteriosclerosis, with inflammatory renal changes. Since that time, these authors have modified their views. Volhard<sup>15</sup> has ceased to believe that the presence of inflammatory glomerular changes distinguishes the combination form from benign arteriosclerosis; he has come to feel that the difference is quantitative, both conditions resulting from ischemia following vascular spasm. Fahr<sup>11</sup> has recognized the fact that renal insufficiency may occur as a slow process during the course of benign sclerosis. Accordingly, he divided the renal changes in essential hypertension into three types: (1) compensated benign sclerosis (pure arteriosclerotic kidney in the stage of compensation); (2) decompensated benign nephrosclerosis, and (3) malignant (specific) nephrosclerosis. He expressed the belief that malignant sclerosis differs from the other forms both pathogenically and histologically. In the malignant form, inflammatory vascular changes in the form of necrotizing arteriolitis, productive endarteritis and periarteritis are present. These lesions are due to a specific toxic factor which is absent in the other forms of hypertensive renal disease.

The separation of the diseases into two distinct pathologic types by Fahr is not accepted by many observers. Löhlein<sup>16</sup> maintained that there is no essential anatomic difference between the two forms. Klemperer and Otani<sup>10</sup> held that malignant hypertension represents an acute and accelerated form of atherosclerosis in which the etiologic agent is the same. Bell and Clawson<sup>17</sup> similarly objected to Fahr's classification and denied the existence of a separate pathogenic form. They divided primary hypertension with renal insufficiency into three types: (1) acute

14. Volhard, F., and Fahr, T.: *Die Brightsche Nierenkrankheit*, Berlin, Julius Springer, 1914.

15. Volhard, F., quoted by Klemperer and Otani: *Arch. Path.* **11**:60, 1931.

16. Löhlein, M.: *Centralbl. f. allg. Path. u. path. Anat.* **28**:209, 1917.

17. Bell, E. T., and Clawson, B. J.: *Arch. Path.* **5**:939, 1928.



hypertension with uremia; (2) chronic hypertension ending acutely in uremia, and (3) chronic hypertension with chronic uremia. The third form is the most common. The renal insufficiency is due to gradual atrophy and fibrosis of the parenchyma following slow vascular occlusion. The first and second forms are frequently associated with necrosis of the arterioles leading to rapid occlusion of the lumens of the arterioles and at times to infarction of the glomeruli. This classification is the most satisfactory because it does not assume an etiologic difference between the three forms and avoids the term malignant hypertension which may be justifiably applied to either the first or the second type.

In all instances of the arteriosclerotic form of flecked spleen in which a fairly complete description of the kidneys was recorded, marked degenerative changes have been noted in the arterioles. These changes infrequently consist only of marked hyaline deposits in the intima, but most of the cases show in addition marked necrosis of the arterioles. These changes are similar to those seen in the small arteries of the splenic follicles; they consist of necrosis of the intima and media, loss of endothelium, hemorrhagic infiltration of the walls and occlusion of the lumen with a hyaline substance. The changes in the larger vessels are arteriosclerotic and consist principally of hyperplastic elastic intimal thickening and connective tissue proliferation.

The glomerular changes are varied in nature. In most cases the glomeruli appear small and frequently show from partial to complete hyalinization. Epithelial and endothelial proliferation has been described in a few instances. Necrosis of portions or of the whole of the glomeruli is noted. This is due to occlusion of the vasa afferentia.

Because of the marked atrophy of the kidneys, it is apparent that many of these cases fall into the second type of Bell's classification, i. e., chronic hypertension ending acutely in uremia. These include the cases described by Lubarsch, Rake<sup>18</sup> (case 1), Feitis<sup>1</sup> (case 1) and Meuret<sup>2</sup> (case 1). A few cases fit more closely into Bell's first type, or acute hypertension with uremia (malignant hypertension of Fahr; accelerated atherosclerosis of Klemperer). The cases of Klemperer,<sup>10</sup> Rake<sup>18</sup> (case 2) and Adolphe<sup>4</sup> (case 3) probably belong to this group. Many cases, however, cannot be classified in either of these groups since anatomic descriptions of the kidneys are lacking.

In six instances, a diagnosis of chronic glomerulonephritis is recorded (Laufer<sup>8</sup> [cases 1 and 2], Kabakaris,<sup>19</sup> Nicod<sup>2</sup> and Adolphe<sup>4</sup> [cases 1 and 2]). Lauder's cases lack evidence of arteriosclerosis and

18. Rake, G.: *Am. J. Path.* **18**:107, 1932.

19. Kabakaris: *Contribution à l'étude des nécroses de la rate au cours de l'urémie*, Thèse de Lausanne, 1930; quoted by Nicod: *Ann. d'anat. path.* **7**:67, 1930.

appear to be examples of subacute and chronic glomerulonephritis showing acute inflammatory vascular lesions resembling periarteritis nodosa. In the other cases, marked vascular lesions consisting of atherosclerosis of the larger vessels, arteriosclerosis and arteriolonecrosis similar to that described were noted. Adolphs, although including chronic recurrent glomerulonephritis in the anatomic diagnosis, stated in the discussion that the renal alterations in his cases 1 and 2 were similar to those described by Fahr<sup>11</sup> in malignant sclerosis and by Stern<sup>20</sup> and Herxheimer<sup>21</sup> in arteriolonecrosis.

The differential pathologic diagnosis between borderline cases of chronic glomerulonephritis and of essential hypertension with uremia is exceedingly difficult. It has been pointed out by Fahr<sup>11</sup> Klemperer and Otani,<sup>10</sup> McGregor<sup>22</sup> and Bell and Clawson<sup>17</sup> that frequently in cases of essential hypertension with uremia inflammatory changes are seen which are indistinguishable from those occurring in glomerulonephritis, varying only in degree of involvement. Crescent formation, proliferation of glomerular endothelium, fusion of tufts of the glomeruli and leukocytic infiltration are frequently seen. Bell expressed the belief that these changes are due to a superimposed inflammatory process. Klemperer and Otani, however, stated that they are due to ischemia following occlusion of the arterioles. On the other hand, in long-standing chronic glomerulonephritis in which the hypertension is severe, changes may occur in the arteries of the kidneys which are indistinguishable from those occurring in hypertension with uremia. Differentiation between the two conditions is based on the prominence of vascular lesions or the inflammatory glomerular lesions; the former are marked in essential hypertension, whereas the latter are more marked and more diffuse in chronic glomerulonephritis. However, there are occasionally borderline cases which are difficult to classify, and not infrequently a subjective element enters into the interpretation of these lesions.

The clinical features in the cases of flecked spleen reported by Adolphs,<sup>4</sup> Nicod<sup>2</sup> and Kabakaris<sup>19</sup> correspond closely to the picture of essential hypertension, since in all cases marked cardiac hypertrophy was observed and the blood pressure was above that usually seen in chronic glomerulonephritis (Nicod, 250 systolic and 180 diastolic; Kabakaris, 210 systolic and 130 diastolic; Adolphs [case 1], 250 systolic). Cerebral hemorrhage, which occurred in Adolph's case is rare in chronic glomerulonephritis, whereas it is commonly observed as a cause of death in essential hypertension.

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20. Stern, M.: *Virchows Arch. f. path. Anat.* **251**:718, 1924.

21. Herxheimer, G.: *Virchows Arch. f. path. Anat.* **251**:709, 1924.

22. McGregor, L.: *Am. J. Path.* **6**:347, 1930.

For these reasons it seems probable that the cases of Kabakaris, Nicod and Adolphs are examples of essential hypertension with marked vascular changes leading to an inflammatory glomerular reaction rather than cases of chronic glomerulonephritis.

Adolphs' case 3 also presents difficulty in classification. The disease lasted only 10 days and terminated in uremia. A diagnosis of early acute diffuse glomerulonephritis was made on the basis of marked diffuse glomerular inflammation. There was also a marked vascular lesion in the form of necrosis and occlusion of the vasa afferentia. Adolphs expressed the belief that the condition was identical with that in the case of early acute glomerulonephritis described by Kuczunski and Hückel, but he admitted that because of the marked arteriolonecrosis it is difficult to differentiate this condition clearly from acute hypertension with uremia.

TABLE 2.—*Age of Patients with "Malignant and Benign Sclerosis" (from Klemperer and Otani) Compared with Age of Patients with the Arteriosclerotic Form of Flecked Spleen*

Age, Years	Malignant Sclerosis	Benign Sclerosis	Flecked Spleen
0-10.....	1}	0}	0}
11-20.....	0}	0}	1}
21-30.....	4}75%	0}29%	0}66.6%
31-40.....	3}	4}	4}
41-50.....	4}	14}	7}
51-60.....	4}	16}	6}
61-70.....	0}25%	22}71%	0}33.3%
71-80.....	0}	6}	0}
Total.....	16	62	18

The age incidence in eighteen cases of the arteriosclerotic type of flecked spleen, when compared with that for malignant sclerosis and benign sclerosis obtained from the studies of Klemperer and Otani (table 2), lends further support to the fact that the renal lesions associated with the arteriosclerotic type of flecked spleen are similar to those in the cases included under the term "malignant or accelerated atherosclerosis." The age distribution of the cases of flecked spleen parallels closely that for malignant sclerosis; 66.6 per cent of the former and 75 per cent of the latter occurred before the sixth decade of life, whereas, 71 per cent of the cases of benign sclerosis occurred in the sixth, seventh and eighth decades.

#### REPORT OF A CASE

*History and Course.*—A woman, aged 45, entered the Colorado General Hospital on Feb. 24, 1933, complaining of progressive weakness and the loss of 40 pounds (18.1 Kg.) in two months. In December, 1932, she contracted a cold which confined her to bed. Since the onset of the cold she had had nocturia and pain in the region of the kidneys. Swelling of the feet and ankles occurred on standing.

The fundi oculi showed star-shaped exudate about the maculae, papilledema and hemorrhage. The heart was enlarged. The blood pressure was 232 systolic and 128 diastolic. The blood chemistry on Feb. 27, 1933, showed: nonprotein nitrogen, 192 mg.; urea nitrogen, 152 mg., and creatinine, 12 mg. On March 29, the non-protein nitrogen was 324 mg., and the urea nitrogen, 256 mg. Hemoglobin was 50 per cent (Dare); the red blood cell count was 2,410,000, and the white cell count, 17,000. Urinalysis showed: specific gravity, 1.010; albumin, 3 plus, and many granular casts and white blood cells. The phenolsulphonphthalein test showed no dye recovered. The Wassermann test was negative.

Ulcerative stomatitis developed and the patient died on March 30, 1933.

*Necropsy.*—The anatomic diagnosis was: arteriosclerosis and arteriolonecrosis of the kidneys; bronchopneumonia; hypertrophy of the heart, especially of the left ventricle; arteriolonecrosis of the heart, suprarenals, pancreas and spleen; serofibrinous pericarditis; ulcerative stomatitis; multiple necroses of the spleen; interstitial fibrosis of the pancreas; generalized atherosclerosis.

The body was poorly nourished; the weight was 90 pounds (40.8 Kg.), and the length, 164 cm. There were areas of ulceration of the buccal and gingival mucous membrane. The heart weighed 595 Gm. There were moderate dilatation and hypertrophy of the right ventricle and marked hypertrophy of the left ventricle. There was a serofibrinous exudate in the pericardial sac. The proximal portion of the anterior descending branch of the left coronary artery showed marked atheromatous deposits and calcification, causing marked reduction in the size of the lumen. The aorta showed a marked degree of atherosclerosis, especially in the lower abdominal portion. The lower lobes of both lungs showed bronchopneumonic consolidation.

The spleen weighed 40 Gm. The capsule showed a moderate degree of hyaline thickening. The surface was roughly nodular. The splenic artery was rigid and tortuous. Its lumen was widely patent. No changes were noted in the splenic vein. The cut surface of the spleen showed many firm, light yellow areas, which stood out in marked contrast to the dark brown-red pulp. They were located, for the most part, near the capsule, the central portion of the spleen being comparatively free. A few of these areas were roughly wedge-shaped; others were irregular, with pointed processes, and a few were small and circular. They varied in diameter from about 2 mm. to 1.5 cm. The larger areas were often connected by narrow bands of light yellow tissue. A zone of hyperemia was noted about some of the larger nodules. The rest of the spleen was firm. The trabeculae appeared increased in thickness.

Both kidneys were shrunken; the left weighed 80 Gm., and the right, 75 Gm. The capsule was firmly adherent to the cortex in places. The surface was finely granular. A few deep depressions were present. There were many small cysts near the surface. The cortex was pale gray-brown. It was markedly reduced in thickness. The renal arteries were rigid and thick-walled.

*Microscopic Examination.*—Spleen: (a) Appearance of the Non-Necrotic Tissue. The trabeculae and the capsule were markedly thickened. The elastic fibers were increased in number and thickness, especially in the trabeculae. The outer portion of the capsule contained few elastic fibers but an abundance of thick collagenous fibers. In places there were small foci of lymphocytes among the collagenous fibers of the capsule and beneath the serous surface. The lymphoid tissue about the central arteries was somewhat diminished in amount. Secondary follicles were absent. The lymphocytes were well preserved and showed no evidence of degeneration. The reticulum of the white pulp appeared thicker and

more compact than normally. The reticular fibers of the red pulp were much coarser and denser than normally. Large numbers of macrophages loaded with hemosiderin were present in the pulp cords and in the sinuses. These cells were irregular in distribution, being closely packed in some areas and few in number in other areas. They bore no relation to the areas of necrosis. In places the venous sinuses were widely patent and distended with red blood cells. In other areas they were small and bloodless.

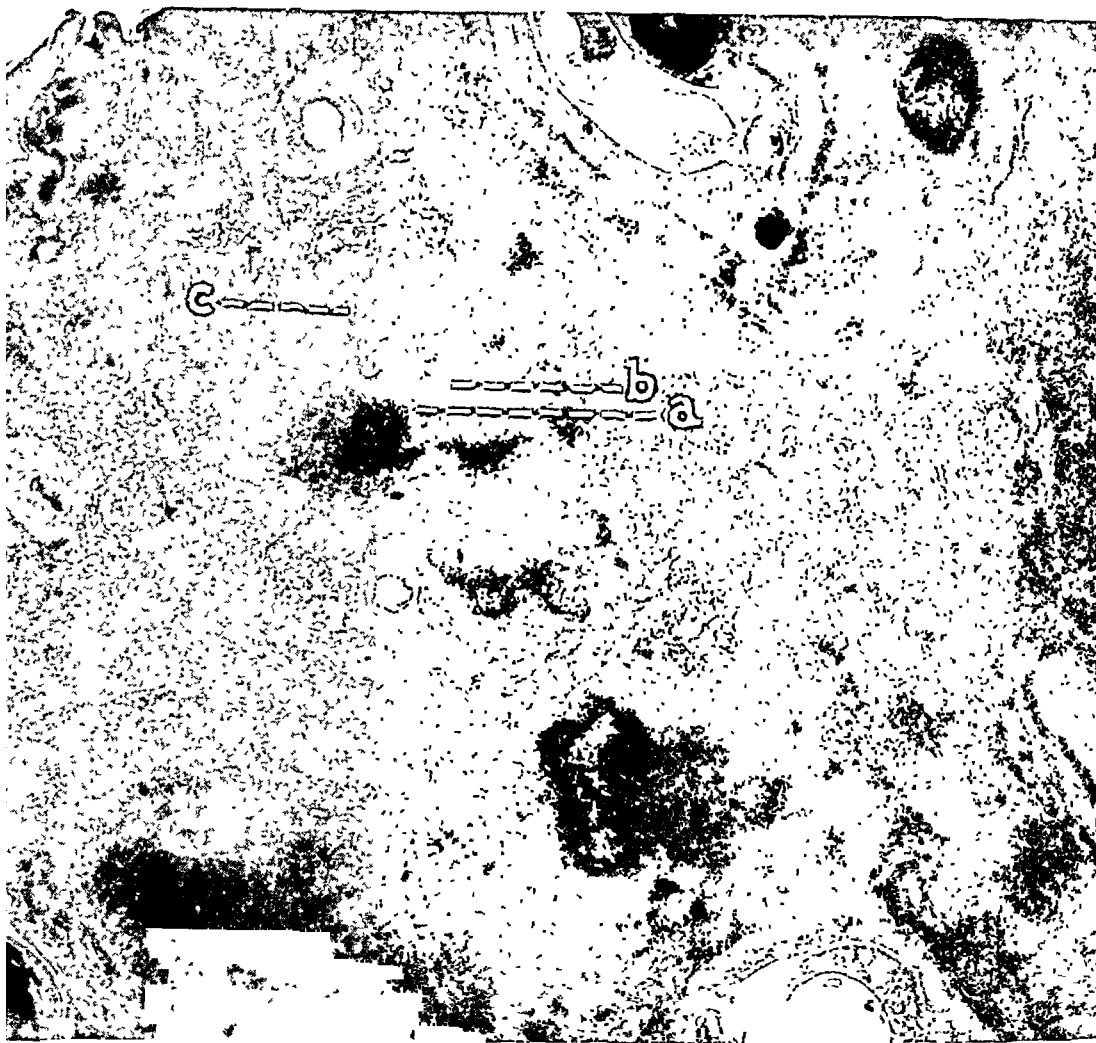


Fig. 1.—A low power photomicrograph through several necrotic foci in the spleen, showing (a) the central zone of necrosis, (b) the intermediate zone and (c) the peripheral zone of hyperemia. Marked arteriosclerotic thickening of the large vessels of the trabeculae is also shown.

(b) Areas of Necrosis. The size and structure of these areas varied considerably. The larger necrotic masses usually showed three fairly well defined zones (fig. 1). Centrally, and occasionally eccentrically, there was a well defined zone consisting of a densely packed mass of degenerated nuclei showing many stages of pyknosis and karyorrhexis. Many of these cells had lobulated nuclei and resembled polymorphonuclear leukocytes. The oxidase stain showed that this zone was loaded with blue-staining granules arranged in irregular small clusters and scat-

tered globules, some of which were from 2 to 3 microns in diameter. This granular mass was embedded in a coarsely reticulated structure which stained light blue with hematoxylin-eosin, light red with Mallory's aniline blue and pale yellow with van Gieson's stain. With Foot's modification of Hortege's silver carbonate method for reticulum<sup>23</sup> a few scattered reticular fibers were found, which were fragmented and thickened and stained poorly. At the periphery of this zone, the necrotic elements were grouped close together and contained clumps of amorphous debris which took a deep red-violet stain with hematoxylin-eosin. Peripheral to this zone there was a rather distinct area of necrosis which varied in thickness. It consisted, for the most part, of a homogeneous mass of necrotic tissue staining less heavily than the central zone and containing fewer and more scattered fragments of nuclei. Included in this mass were fragments of trabeculae, blood vessels and lymph follicles, the outlines of which were fairly distinct. With aniline blue a framework of thick, pale blue-staining fibers could be made out. With scarlet red, there was an abundance of fat in the form of small isolated droplets and small clusters of droplets of irregular size. Fat was much more abundant in this zone than in the central zone. The third, or peripheral, zone was an area of hyperemia which merged gradually into the intermediate zone. The inner portion contained many polymorphonuclear cells and large macrophages loaded with cellular debris. There was no evidence of connective tissue proliferation. With a stain for hemosiderin a few isolated granules were seen in the central and intermediate zones, and many coarse clusters in the peripheral zone. The oxidase stain (Schultze) showed fewer and more scattered granules in the intermediate zone than in the central zone. The peripheral zone contained numerous cells giving the oxidase reaction. Some of the larger areas of necrosis were composed of conglomerate clusters of smaller areas, since some of them showed two or more central zones.

In addition to these areas of necrosis, there were many small necrotic areas which did not show the three zones described. Some areas showed a structure similar to that of the central zone surrounded by an area of hyperemia. Other areas showed early necrosis of the parenchyma, with a diffuse infiltration of polymorphonuclear leukocytes. Small foci of early necrosis of the parenchyma showing little or no cellular reaction were present. These areas bore no relation to the trabeculae or to the lymphoid elements.

(c) Changes in the Arteries. The small central arteries of the white pulp showed striking changes, especially near the areas of necrosis. Replacing the intima and a large portion of the media, there was a dense layer of a homogeneous substance staining a light pink-lavender with hematoxylin-eosin. This amorphous material was sometimes arranged in the form of irregular wavy bands which were roughly parallel to the wall of the vessel. In many of these vessels the endothelium was lacking and the lumen was completely filled by a hyaline substance, embedded in which there were occasional deformed nuclei and red blood cells (fig. 2). With van Gieson's stain, this hyaline material was light yellow with orange streaks, and with Mallory's aniline blue, a homogeneous dark cloudy blue. Weigert's stain for fibrin failed to reveal a positive color reaction. With the elastic tissue stain, the inner elastic membrane appeared to be embedded in the hyaline substance, stained poorly and in many places was split into two or more indefinite layers. Many of the fibers were discontinuous and fragmented.

The precapillary arteries of the red pulp occasionally showed changes which were similar to those of the arteries in the white pulp. Many of these vessels were converted into a hyaline mass containing nuclear fragments. This hyaline material

23. Foot, N. C., and Menard, M. C.: Arch. Path. 4:211, 1927.

occluded the lumens of many of the arteries. However, these changes were not as widespread or as marked as the changes in the arteries of the follicles.

The large central arteries of the lymphoid sheath and the small arteries of the trabeculae also showed striking changes. Hyaline material similar to that seen in the smaller vessels was present in many of these arteries, involving the intima and the inner portion of the media and frequently occluding the lumen. Occasionally red blood cells and leukocytes were seen in the outer layers of these vessels. In addition to these changes, the arteries of the trabeculae showed a marked degree of atherosclerosis. There was marked connective tissue proliferation, causing marked diminution in the diameter of the lumen in many of the arteries. The inner elastic membrane was frequently thickened, and often it showed two or more layers. Heavy deposits of calcium were present in the intima and frequently in the media

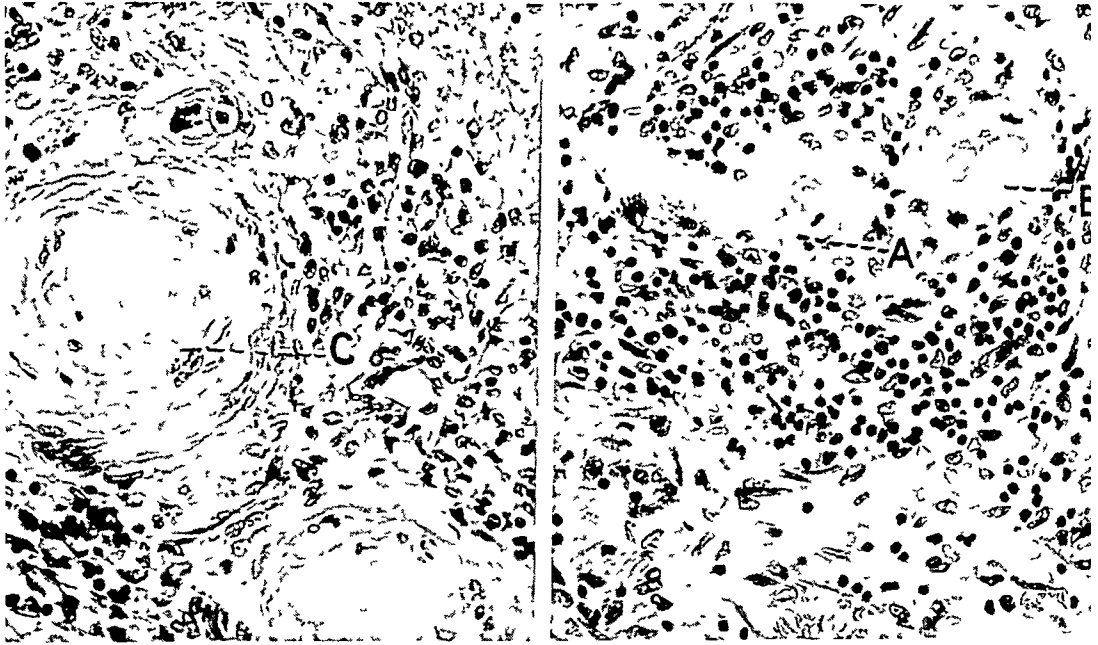


Fig. 2.—Necrosis of the inner wall of two small arteries (*A* and *B*) and one large artery (*C*) of the lymphoid follicle of the spleen. The lumens of these arteries are completely filled with a hyaline substance.

At times the calcium formed a complete thick ring in the wall of the artery. Calcification of the internal elastic membrane was seen. Some of the thickened, calcified vessels showed marked necrosis of the intima and media, with extravasation of blood between the necrotic layers and infiltration of polymorphonuclear cells. In the lumens of these vessels there were clumps of coarse granules which stained dark violet with hematoxylin-eosin and black with von Kossa's stain for calcium. In places, as the result of partial destruction of the inner wall of the vessels, the granular deposit in the lumen was continuous with the calcium deposits in the intima and media (fig. 3). About these granules were necrotic cells containing droplets of fat and red blood cells. A few of the vessels of the trabeculae showed partially organized thrombi occluding the lumen. With scarlet red, fat was abundant in the intima of the trabecular arteries which showed connective tissue hyperplasia of the intima. In many of the necrotic vessels of the follicles, small droplets of fat were seen in the amorphous hyaline material described.

In a few of the small veins, the lumen was partially closed by platelet thrombi. Most of the veins, however, appeared normal.

**Kidneys:** Many of the glomeruli were enlarged. Occasionally there was fusion of one or more tufts with the Bowman capsule. In some of the glomeruli, there was marked swelling as well as an increase in the number of endothelial cells and a moderate increase in polymorphonuclear leukocytes in the lumens of the capillaries. There was no evidence of crescent formation. With the azan-carmine aniline blue stain the basement membrane of the glomerular tufts appeared thickened and tortuous. Many of the loops contained intracapillary fibrils which formed a distinct network. In places, the capillaries were filled with a dense blue-staining

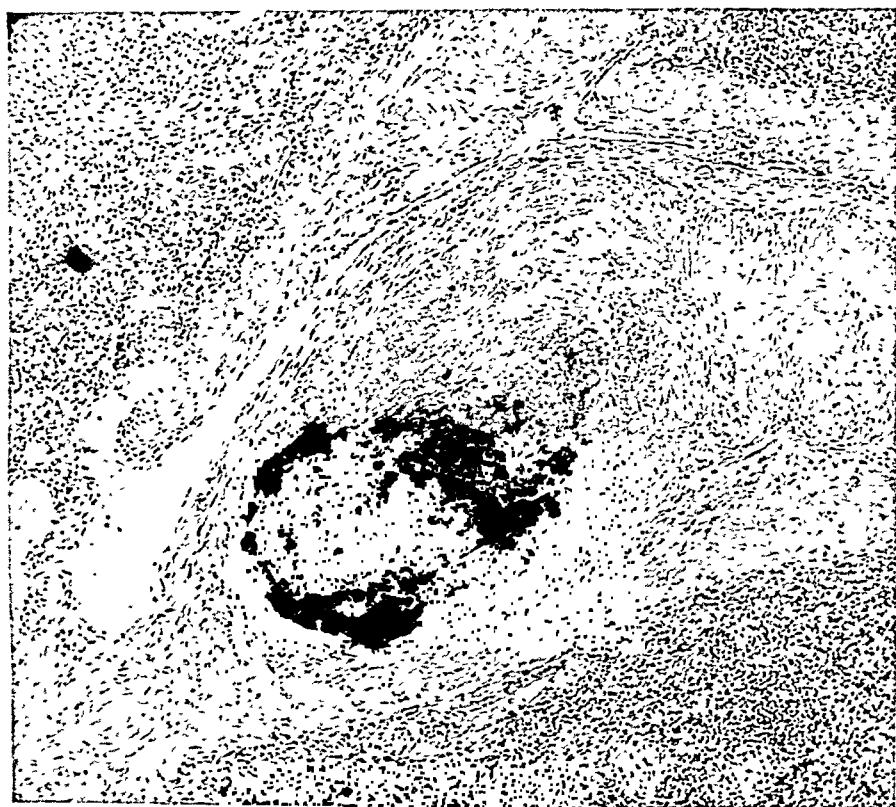


Fig. 3.—A trabecular artery, showing necrosis of the inner wall with extravasation of red blood cells and infiltration of leukocytes. There is desquamation of the necrotic tissue and calcium into the lumen of the vessel.

fibrillar material which completely occluded the lumen and was fused with the capillary basement membrane. Some of the glomeruli showed a moderately thickened capsular basement membrane. Some of the glomeruli showed necrosis of the tufts, and a few were converted into an amorphous granular mass containing necrotic nuclear fragments. Hyaline droplets were noted in many of the swollen endothelial cells. A large percentage of the glomeruli showed varying degrees of hyalinization.

The interlobular arteries showed marked thickening of the intima, owing to an increase in thickness and duplication of the internal elastic membrane and to connective tissue hyperplasia. There was a marked subintimal deposit of hyalin in the afferent arterioles, causing a marked diminution in the size of the lumen.



Other arterioles showed changes similar to those in the small arteries of the spleen, namely, marked necrosis of the walls with the formation of a hyaline material which frequently occluded the lumen. Embedded in the hyaline substance were fragmented nuclei. Scarlet red showed numerous fat droplets in the hyaline masses. There was no evidence of periarteritis or endarteritis. The tubules showed marked degenerative changes. Many of them contained large plugs of leukocytes. The cytoplasm of the convoluted tubules contained many hyaline droplets. Atrophy of the parenchyma was marked, with replacement by connective tissue containing many lymphocytes.

**Pancreas:** Many of the small arteries of the pancreas showed changes similar to those seen in the follicular arteries of the spleen. However, the lesions were not as severe or as extensive. There were occasional medium-sized arteries which showed marked atherosclerosis and degenerative changes similar to those seen in the spleen. A moderate degree of interstitial fibrosis was present.

**Heart:** There was replacement of areas of muscle bundles with hyalinized connective tissue. In the adventitia of many of the small arteries there was infiltration with polymorphonuclear cells and a few lymphocytes and plasma cells. An occasional small artery showed necrosis and hyalinization with occlusion of the lumen similar to the change seen in the spleen and kidneys.

**Other Organs:** In the capsule of the suprarenal glands, small arteries containing a thick hyaline band beneath the intima were occasionally seen. No changes were observed in the small vessels of the brain. Section through the lower lobes of the lungs showed early lobular pneumonia. The vessels of the lungs appeared normal.

#### COMMENT

The renal changes in this case correspond to the second type of Bell's classification, i. e., chronic essential hypertension terminating in acute uremia. Because of the marked cardiac hypertrophy and renal atrophy, it is justifiable to conclude that the hypertension had existed for a long time. The disease was not clinically manifest until about two months before death, following an acute infection of the upper respiratory tract. Symptoms of uremia and of albuminuric retinitis rapidly developed, and the patient died in uremic coma. The outstanding renal changes, in addition to the long-standing atherosclerosis, was marked necrosis of the vasa afferentia. Arteriolitis such as Fahr<sup>11</sup> observed in malignant hypertension was absent. The inflammatory lesions in the glomeruli were not diffuse but focal and consisted of changes which were similar to those seen in glomerulonephritis. Necrosis of the glomeruli due to closure of the vasa afferentia was also noted.

Symptoms referable to involvement of the spleen were lacking. Multiple infarct-like areas occupied about one third of the organ. The appearance of the spleen was similar to that described in case 2 of Feitis.<sup>1</sup> The marked irregularity in the shape of the necrotic foci was due to the confluence of small areas of necrosis. Because of this, the microscopic structure of these infarcts was more complex than that due to embolic occlusion of large vessels. Moreover, they differed from embolic infarctions in that most of the necrotic areas were heavily infil-

trated with leukocytes. This difference may be accounted for by the fact that in embolic infarction the large areas of anemic necrosis are produced by the closure of large arteries, whereas in flecked spleen the necrosis is due to the closure of many small arteries. Because of the richness of the collateral vascular supply about these small areas of infarction in flecked spleen, the degenerative changes, in all probability, proceed slowly and incite a marked response of polymorphonuclear leukocytes and macrophages. The larger areas, in which three well defined zones can be distinguished, were probably caused by occlusion of the larger arteries of the trabeculae. The vascular changes were similar to those seen in the kidneys, although more extensive. Two types of lesions were observed: (1) marked atherosclerosis of the medium-sized and large arteries of the trabeculae, with marked hyperplasia of the intimal elastic fibers and connective tissue hyperplasia, marked fatty degeneration and calcification; (2) necrosis of the trabecular arteries and especially of the arteries of the follicles, leading to occlusion of the lumens. In the medium-sized trabecular arteries were seen peculiar changes. There was marked necrosis of the walls, with desquamation of the inner wall into the lumen of the vessel. Because of the heavy calcium deposit and the atheromatous intimal changes, clumps of this material were seen occupying the lumens of many of these vessels. Spier<sup>24</sup> described this violet-staining material in the lumens, but he claimed that the substance is fatty, since it reacted positively to specific stains for fat. However, this material also took von Kossa's silver stain for calcium. On staining with scarlet red and counter-staining with methylthionine chloride, U. S. P. (methylene blue), an abundance of fat droplets was present in addition to a large amount of calcium which stained dark blue. In some of the arteries, the granules of calcium were still in direct contact with the heavy calcium deposits in the intima and media (fig. 3), indicating their origin from this source. Spier's view, that this material is not a form of thrombus but a product of degeneration of the wall of the vessel, was well taken. However, contrary to her observations and in accordance with those of Feitis, a few of the trabecular vessels showed thrombi which are partially organized.

The nature of the material occluding the smaller arteries is difficult to determine. It consisted of a homogeneous, amorphous substance, which stained light pink-violet with hematoxylin-eosin and which was arranged at times in the form of thick, irregular laminae lying parallel to the walls of the vessel. It stained dark blue with Mallory's aniline blue and pale yellow to orange with van Gieson's stain. Wright's fibrin stain and Mallory's aniline blue stain failed to show any evidence of fibrin. The marked similarity of this substance to the subintimal deposits

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24. Spier, B.: Frankfurt. Ztschr. f. Path. **41**:160, 1931.

of hyalin commonly seen in these vessels strongly suggests a similar origin. The nature of this hyaline deposit is still not understood. Heuck<sup>25</sup> expressed the belief that it consists of coagulated albuminous substances. Whether it is in a fluid or a solid state has not been determined. It is conceivable that this hyaline substance may swell and accumulate rapidly in acute forms of hypertension and that following necrosis of the endothelial lining it may completely occlude the lumen of the vessel.

Feitis<sup>1</sup> considered three possibilities in an attempt to account for the peculiar necrosis of the spleen: (1) that the necrosis is due directly to occlusion of the smaller and medium-sized arteries; (2) that the changes are due to a combination of two factors, a toxic injury to the parenchyma and partial or complete occlusion of the arteries, and (3) that the lesion is due to toxic injury of the parenchyma, the vascular changes being secondary. He concluded that the first theory is the most plausible.

It is evident that in the case reported here the lesions in the arterioles and the larger arteries were entirely independent of the parenchymal injury since similar changes are noted in the pancreas, heart and kidneys. Material found in the follicular arteries and the larger arteries of the trabeculae can hardly be considered as derived from the necrotic parenchyma, since the two substances are morphologically different. The substance filling these arteries appeared to be derived from the walls of the vessels except in a few arteries of the trabeculae which contained thrombi. Although a toxic condition is associated in cases of flecked spleen, it is doubtful that this factor plays a direct part in the production of the infarct-like areas of necrosis of the spleen, as necrosis of this type is not seen in pronounced uremia of essential hypertension without marked lesions of the small splenic vessels.

It is difficult to account for the rarity of flecked spleen. Whereas it has been shown that the condition occurs in by far the majority of cases in association with essential hypertension terminating in uremia, the association of the two conditions is rare. Klemperer and Otani<sup>10</sup> found but one instance in eighteen cases of malignant nephrosclerosis. Bell and Clawson<sup>17</sup> found no example in thirty-six cases of essential hypertension associated with uremia. On the other hand, the vessels of the spleen frequently show changes in generalized atherosclerosis and hypertension (Fishberg,<sup>26</sup> Spier<sup>24</sup> and others). It was shown by Herxheimer,<sup>27</sup> in a study of the spleens of 1,140 normal persons, that hyaline degeneration of the small vessels occurs as early as the age of 10 years and

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25. Heuck, W.: München. med. Wchnschr. **67**:535, 1920.

26. Fishberg, A. M.: Arch. Int. Med. **35**:650, 1925.

27. Herxheimer, G.: Berl. klin. Wchnschr. **54**:82, 1917.

increases during life. Obviously marked narrowing of the small arteries under normal conditions and in most cases of arteriolosclerosis does not produce necrosis of the parenchyma. The rich vascular supply of the pulp evidently compensates for the marked restriction in size of the peripheral arterial circulation in the majority of cases. It is only in rare instances in which there is extensive occlusion of the small arteries that the lesions of flecked spleen occur.

#### SUMMARY

Flecked spleen of Feitis is an extremely rare condition characterized by nonembolic multiple areas of anemic necrosis.

The necrosis is due to occlusion of the splenic arteries of small and medium size. On the basis of the nature of the vascular lesion, three types of flecked spleen are recognized: (1) arteriosclerotic, (2) arteritic and (3) thrombotic. The arteriosclerotic form is the most common, comprising all but three of the twenty-one cases of flecked spleen described in the literature.

The arteriosclerotic form of flecked spleen is associated with renal lesions of hypertension, which in most cases produce death from uremia. The thrombotic form, described in a report of a case of eclampsia, is associated with multiple necroses of the kidney. The arteritic type is described in association with two cases of glomerulonephritis.

Flecked spleen should be differentiated from multiple necroses of the malpighian corpuscles associated with acute infectious diseases, as the pathologic changes and pathogenesis in the two conditions are dissimilar.

A case of flecked spleen is described in which the anatomic and histologic changes were similar to those reported by Feitis. The kidneys show the lesions of an advanced stage of arteriosclerosis accompanied by arteriolonecrosis.

# STRUCTURAL CHANGES IN THE GRANULAR LAYER OF THE CEREBELLUM

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The purpose of this study was to determine if, among the various pathologic lesions of the cerebellum already described, there could be added another condition which has been recorded under the terminology of "conglutination of the granular layer," and if other pathologic changes of the same layer could be found.

Conglutination consists of a clumping of numerous cells of the granular layer which lose their individuality and form irregular and solid masses of elements undergoing a more or less advanced degeneration. Such pathologic changes, which have already been described by Ferraro and Morrison<sup>1</sup> in various experimental conditions, have been investigated in the course of this study in animals and in various human pathologic conditions.

For practical purposes the cortex of the cerebellum may be divided into: (1) the outer layer or molecular layer which contains few medullated fibers and few nerve cells; (2) the nuclear layer, better known as the granular layer; (3) the layer of Purkinje cells, formed by flask-shaped elements situated between these two other layers.

The granular layer is formed by nuclei of cells rather closely packed together, with here and there clear spaces between them, and forming what is known to some authors as islands or glomeruli of the cerebellum. Scattered throughout are larger cells known as Golgi cells. Hence the two chief cells of this layer are granular cells and Golgi cells.

The granular cells are karyochrome in type and are rather small, about 0.7 micron in diameter. Their nucleus is rather large and surrounded by a cytoplasmic ring which is fairly narrow. Each cell has from three to six fairly short dendrites which terminate close to the cell body in what may be called a limited arborization. Where these arborizations meet, there results an apparent empty space known as dendritic islands. The axons of the granular cells extend through the

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From the Department of Neuropathology of the New York State Psychiatric Institute and Hospital.

1. Ferraro, A., and Morrison, R.: *Psychiat. Quart.* **3**:506, 1928.

superficial, deep and intermediate levels of the molecular layer, from all of which positions they give rise to the typical T-shaped and at times Y-shaped branches. They are without myelin sheaths. The type of synaptic union between the axons of the granular cells and the dendrites of the Purkinje cells is the cruciform variety and thus brings about extensive Purkinje synaptic connections.

Three varieties of stellate cells of Golgi are recognized in the granular layer, the most important being the stellate corpuscles.

In the embryonal development of the cerebellum in man as well as in animals there is besides this granular layer the external granular layer which is to be found external to the molecular layer. The cells of this external molecular layer are known as the cells of Obersteiner. They gradually disappear, and in man two years after birth they are no longer present.

In animals, more particularly in cats, this external layer of granules which is pronounced immediately after birth gradually disappears, and in cats 6 weeks old the layer is practically absent.

Three kittens and two adult cats were examined for purposes of control, and while the new-born animals showed a considerable number of cells in the external granular layer, the animal 5 weeks old showed a marked decrease in the number of cells and width of the layer. The external granular layer in the new-born cat is apparently formed by cells running in two directions: in the outer layer the cells form rows which are perpendicular to the inner layer, the course of which is parallel to the external surface of the molecular layer (fig. 1).

Though even in normal conditions the cells of the internal granular layer have a tendency to form glomeruli as already mentioned (fig. 2), there is no evidence of any abnormal clumping, fusion or conglutination of the elements, comparable with the pathologic picture in "conglutination."

It must be mentioned, however, that another adult animal that was used for control disclosed a slight amount of conglutination, which fact led me to the belief that in this particular case a pathologic process of unknown nature must have been active in the animal and caused pathologic changes in the granular layer. That such must be the case is also indicated by the fact that in a large series of other animals, though they were in a pathologic condition, conglutination was entirely absent.

In order to establish if postmortem changes could determine the occurrence of conglutination in the granular cells of the cerebellum,

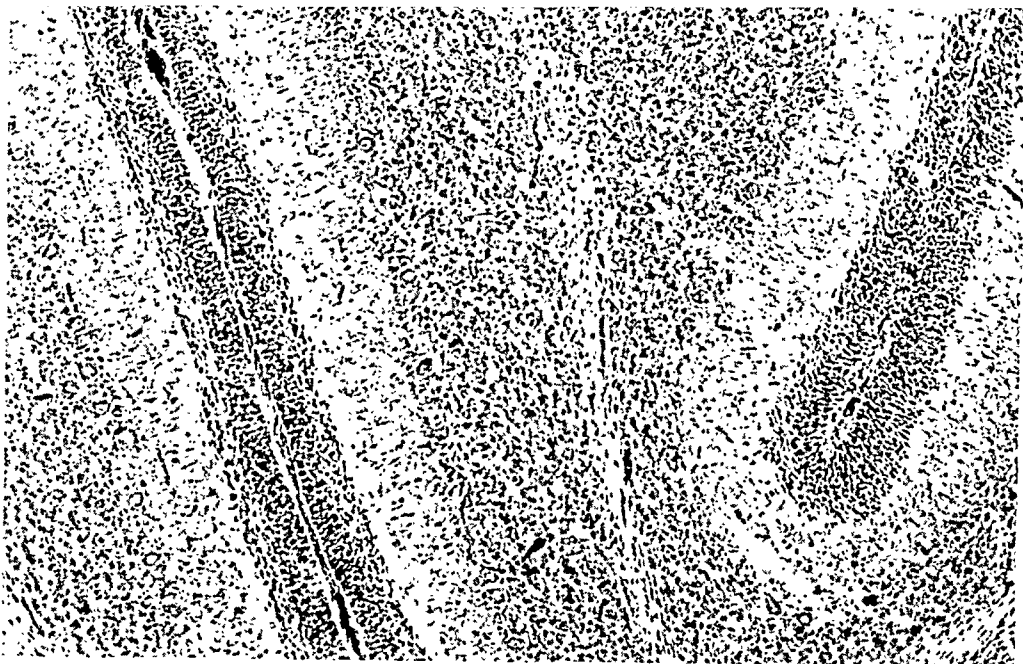


Fig. 1.—Presence of an external granular layer in a new-born cat. Note the existence of two rows of cells perpendicular to each other. Nissl stain.

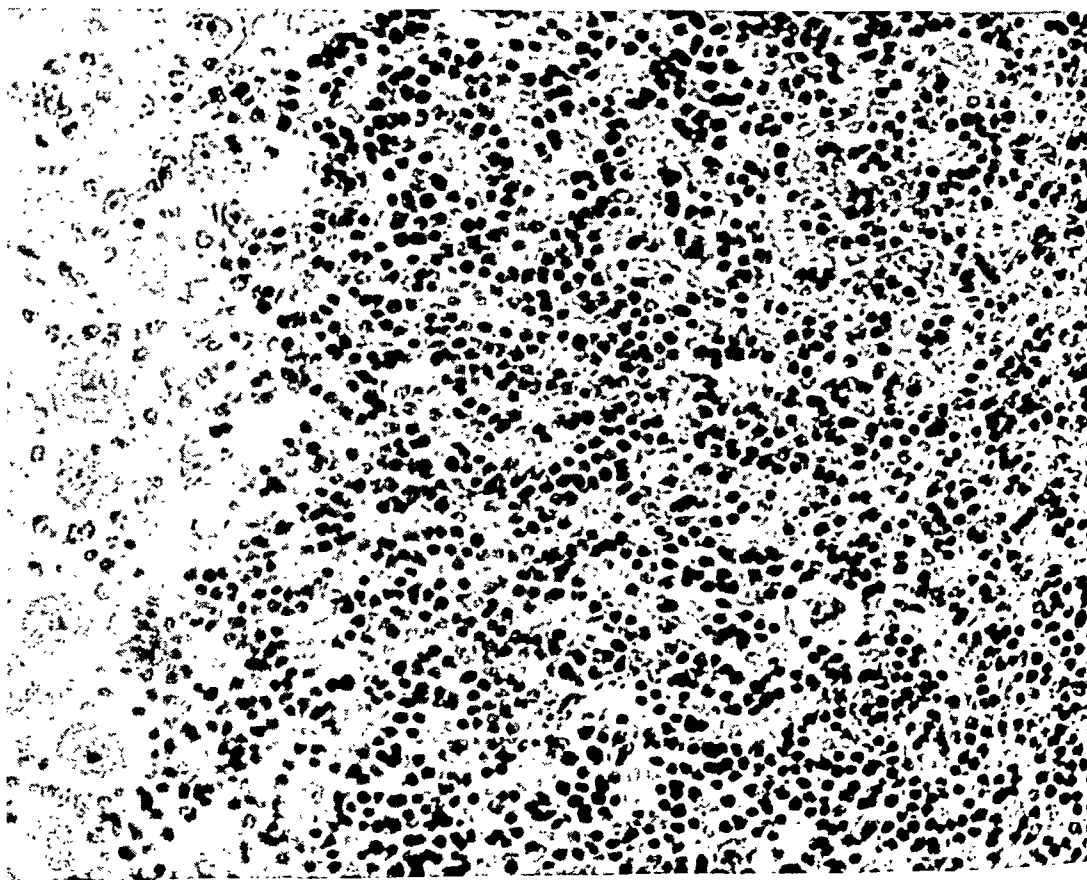


Fig. 2.—Normal appearance of granular layer in an adult cat. Note the tendency of formation of cerebellar glomeruli in which, however, each cell maintains its own individuality. Nissl stain.

partial removal of the cerebellum was done immediately after the death of a cat, twenty-four and thirty-six hours following death. The purpose was to see if the time between death and fixation would have any influence in establishing conglutination. It was found that none of the specimens showed any conglutination.

## EXPERIMENTAL WORK

In investigating the reaction of the granular layer to various pathologic conditions, twelve cats were subjected to experimental lead poisoning (table 1). In the first group of animals lead carbonate was administered orally; in the second group lead was given intravenously

TABLE 1.—*Experimental Lead Poisoning*

Group	Cats	Total Weight Drugs, Gm.	Mode of Administration	Single Dosage	Days Before Death
1	1	57.0	Lead carbonate orally, daily	0.74 Gm.	78
1	2	44.0		0.71 Gm.	62
1	3	49.0		0.84 Gm.	58
2	13	9.0	Intravenously, 15 cc. of 1% solution lead acetate and orally as lead carbonate	1.5 cc.	33
2	12	17.0		1.5 Gm.	21
3	5	0.60	Acetate of lead, intravenously and daily	20 cc. of 1% sol.	3
3	6	0.40		10 cc.	4
3	7	0.22		2 cc.	11
4	8	0.15	Acetate of lead, 1% solution, intra- venously	5 cc.	3
4	9	0.15		3 cc.	5
4	11	0.20		2 cc.	10
4	12	0.20		10 cc.	2

as lead acetate, and by mouth as lead carbonate; in the third group lead acetate exclusively was given intravenously. The animals in these groups were allowed to die from the effect of the poisoning, whereas in the next group, in which lead acetate was also used intravenously, the animals were killed after fixed periods of time in order to study the early stage of reaction.

As a result of the study of these twelve cats, I reached the conclusion that in the long-standing cases, particularly in the three animals of the first group, conglutination was present, but the main pathologic change was the rarefaction of the granular layer. As a matter of fact, the number of cells present in this layer was considerably less than normal. This disappearance of cells, as represented in figure 3, seems to be parallel with the disappearance of cells of the Purkinje layer which also suffer considerable loss from lead poisoning. In the cases of group 2 conglutination was present in the two animals, and a slight



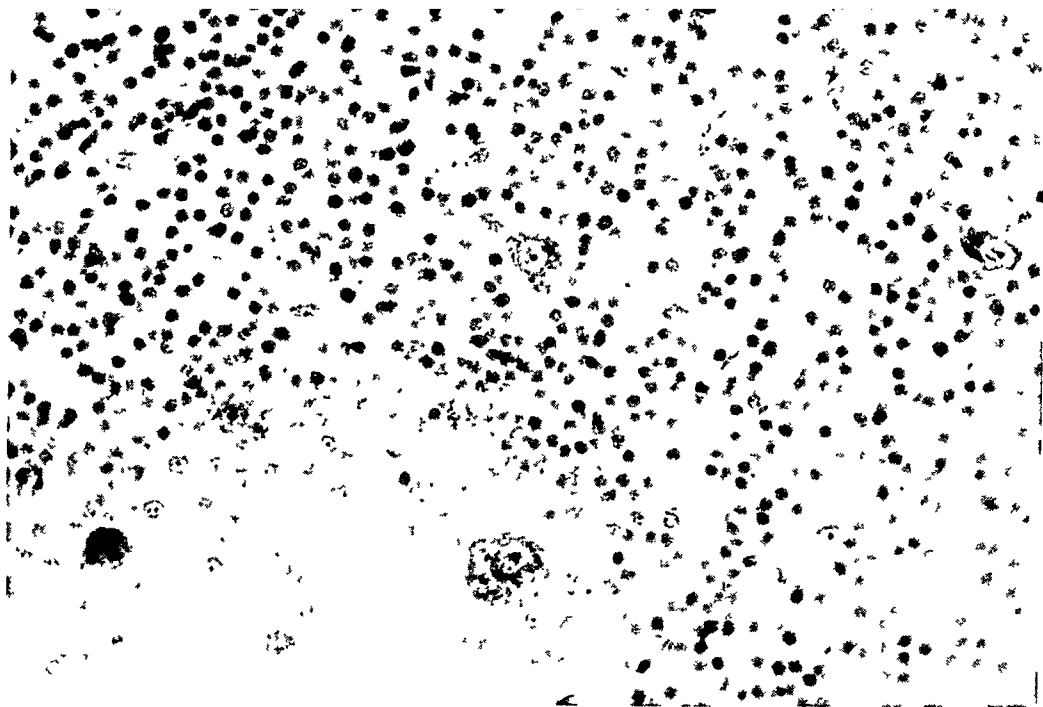


Fig. 3.—Rarefaction of the granular cells resulting in a widespread distribution of the remaining elements. Nissl stain



Fig. 4—Marked conglutination of the granular cells in indole and histamine poisoning. Nissl stain.

rarefaction was also noticeable. In group 3, the third animal, cat 7, showed slight conglutination associated with a slight loss of granular cells. In the fourth group conglutination was evident, particularly in cat 8.

Altogether, it seems that the more acute the lesion and the larger the amount of lead the more conglutination is evident, whereas the longer the animal survives the more the destruction of granular cells

TABLE 2.—*Experiments with Indole, Histamine, Potassium Cyanide Poisoning*

Cats	Weight in Kg.	Total Amount Given	Mode of Administration	Single Dosage	Days Before Death
3	3.25	0.5 Gm. indole	} Hypodermically in olive oil (3 injections of 100 mg. each and 73 injections of 150 mg. each)	0.25 Gm.	2
6	3.6	11.25 Gm. indole		.....	96
2	3	1 Gm. indole	Hypodermically in olive oil	0.5 Gm.	2
E	3.5	2.6 Gm. indole 0.7 Gm. histamine	Hypodermically in olive oil	100 mg. 25 mg.	41
D	3	1.2 Gm. indole 0.3 Gm. histamine	Hypodermically in olive oil	100 mg. 25 mg.	12
8	4.4	6.6 Gm. indole 2.6 Gm. histamine	Hypodermically in olive oil	100 mg. 40 mg.	92
C	3.2	13 Gm. histamine	Hypodermically	Increasing from 5 to 80 mg.	82
9	...	1.5 Gm. indole 10 mg. potassium cyanide	Hypodermically	150 mg. 0.5 mg. in increasing doses to 2.5 mg.	13
9	4.2	600 mg. indole 7 mg. potassium cyanide	Hypodermically	100 mg. 2.5 mg.	6
Dinky	3	516 mg. potassium cyanide	Hypodermically	2.3 mg. in increasing doses to 28 mg.	37

takes place, thus upholding the conception that the cells undergoing conglutination later degenerate and disappear, the process resulting in a scarcity of the granular element.

The next group consists of eleven cats poisoned with histamine, indole, potassium cyanide or a combination of any two of these drugs (table 2).

Among the cats poisoned with indole a certain amount of conglutination was found in the granular layers, particularly in cat 3. In the



Fig. 5.—Rarefaction of the granular cells in a case of potassium cyanide poisoning. Nissl stain.

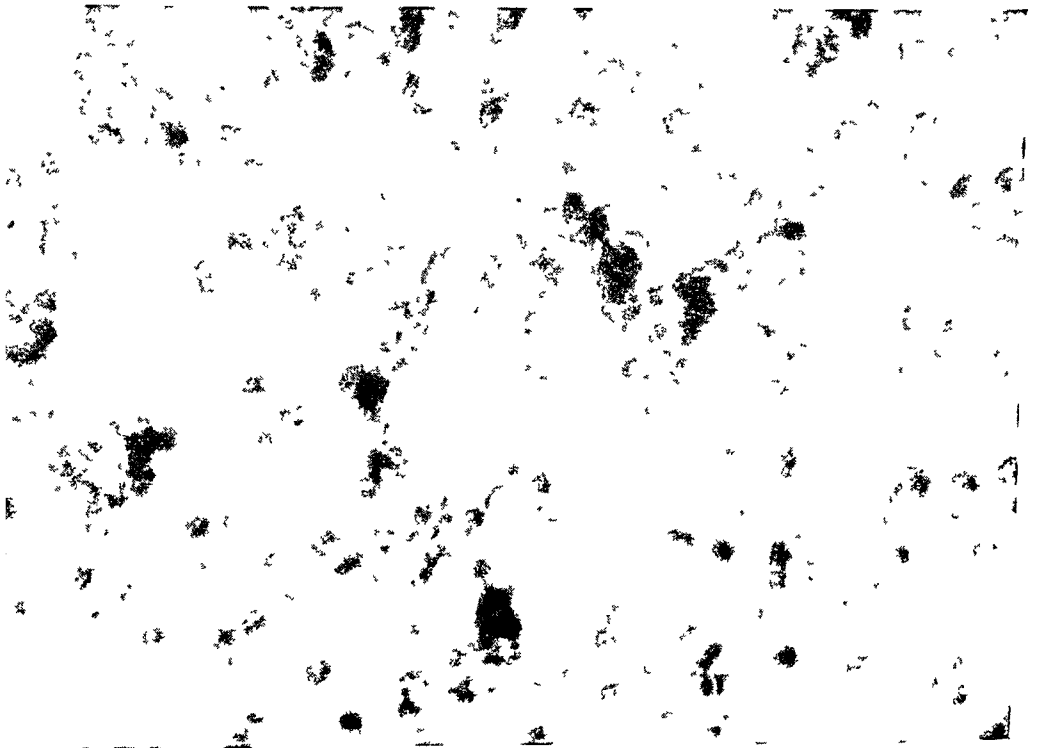


Fig. 6.—Conglutination of the granular layer in a case of illuminating gas poisoning. Nissl stain.

group poisoned with indole and histamine, the conglutination was much more pronounced, particularly in cat D (fig. 4). In cat C, which received 13 Gm. of histamine subcutaneously, there was an evident conglutination associated with loss of granular cells. In the group in which indole and potassium cyanide are used conglutination and disappearance of cells were found. In the cat, Dinky, which received 516 mg. of potassium cyanide, conglutination was not present in any noticeable amount, but there was considerable loss of cells in the granular layer (fig. 5).

Another experimental group included two cats, L and M, which died from provoked inanition. Cat L, which died on the forty-first day, was allowed to drink water during the experiment. This animal did not show conglutination, whereas cat M, which died on the fifty-second day of the experiment and which was not allowed to have any water, showed a certain amount of conglutination of the granular cells.

Rabbits were also studied under experimental conditions of repeated illuminating gas poisoning and of repeated intravenous injections of hypotonic solution.

Of the first group, five animals were examined after various periods of gassing through a glass chamber in which the animals were placed and into which two tubes carried illuminating gas and oxygen, respectively. In these five animals there were evident traces of conglutination. In the most advanced cases there was also a destruction of granular cells. Figure 6 illustrates conglutination in an animal which had been gassed eight times in thirty-five days. It can be seen that while some of the elements are fused together, others are in a more or less advanced state of degeneration.

Seven rabbits were treated with intravenous injections of hypotonic solution. They received various and repeated amounts of the hypotonic solution, from 35 cc. of distilled water to 950 cc. The number of injections varied from one in the rabbit which received 35 cc. to twelve in the rabbit which received 950 cc. The injections were given under sterile precautions in the auricular vein at intervals of twenty-four hours. The animals were killed after the last injection. In this series no conglutination was found. Conversely, there was a tendency of the cells of the granular layer to swell up and acquire a more marked individuality than normally. Because of the hydropic condition of the single elements, the cells appear to be stained less deeply than normally. Small vacuoles were noticeable in some of the cells (fig. 7).

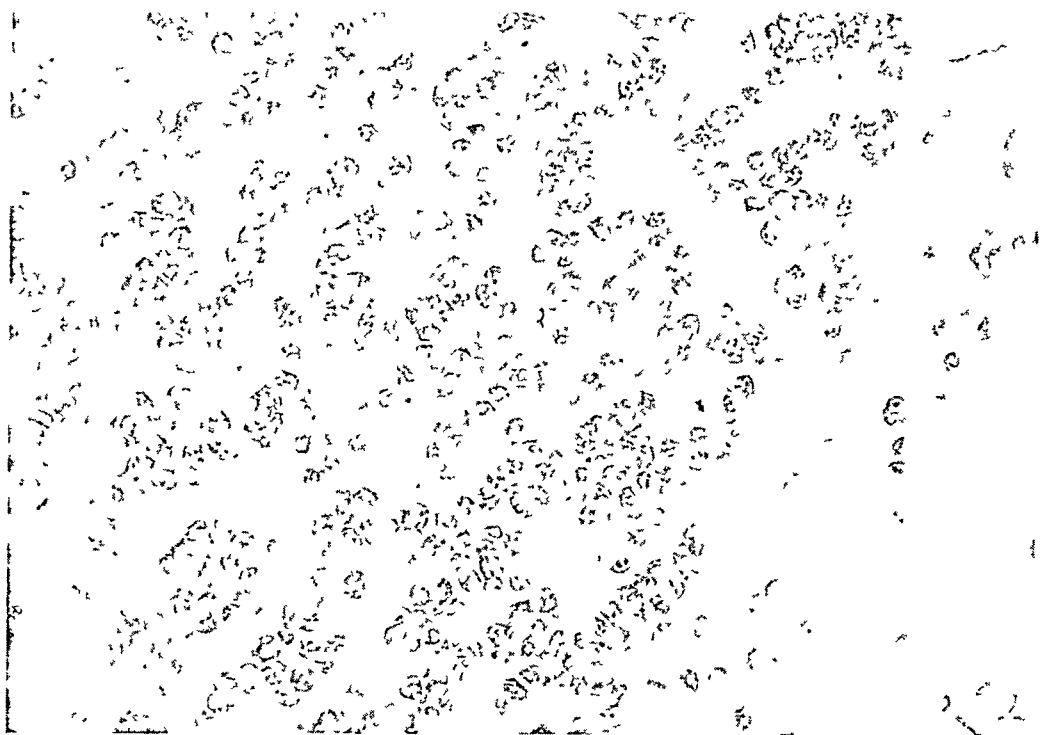


Fig. 7.—Swelling and vacuolation of the granular cells of the cerebellum following intravenous injection of hypotonic solution in rabbits. Hematoxylin and eosin stain.

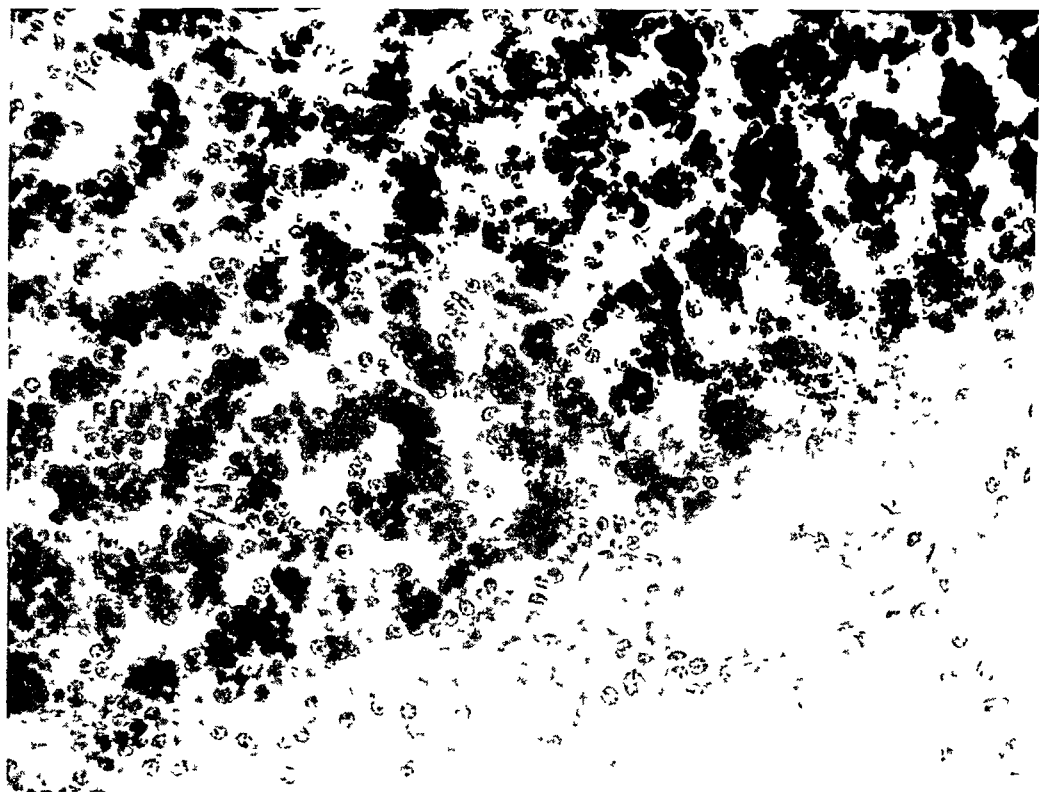


Fig 8.—Conglutination in a case of dementia paralytica Nissl stain

## HUMAN MATERIAL

*Normal Control.*—In order to study the distribution of these granular cells in normal persons, three cases of sudden accidental death were studied. Conglutination was not found.

*Pathologic Material.*—In the following cases the histologic changes in the granular layer were studied: dementia praecox, four cases; dementia paralytica, five; cerebral syphilis, four; Huntington's chorea, three; Schilder's disease, two; various types of acute meningitis, five; electrocution, two; epidemic encephalitis, two.

In the cases of dementia praecox there was no conglutination except that in one case there were indications of conglutination in a few areas.

In dementia paralytica the results were not constant; in some instances conglutination was absent, and in other instances present and even marked. Figure 8 shows definite conglutination, though some of the cells that come together still have preserved their individuality.

In cerebral syphilis no conglutination was present as a rule but rather a rarefaction of the cellular elements.

In epidemic encephalitis conglutination was present in one of the two cases but not uniformly or in severe degree.

In the two cases of Huntington's chorea loss of granular cells was found but no conglutination.

In the two cases of Schilder's disease there was no conglutination, but a definite loss of granular cells in association with a considerable amount of gliosis which was evident in the intercellular spaces, thus making the loss of cells more discernible (fig. 9).

In the group of septic meningitis all the cases showed more or less well marked conglutination.

In the two cases of death from electrocution no conglutination was found, but conversely a certain amount of swelling in the individual cells which appear more distinct though poorly stained. The condition resembles somewhat the condition described following the injection of hypotonic solution in rabbits (fig. 10).

## SUMMARY

In addition to pathologic changes of the other layers of the cerebellum, a particular type of structural change has been found to be quite common in the granular layer of the cerebellum.

The granular cells of the cerebellum may collect or fuse into smaller and larger clumps in which the individual cells lose their outline and

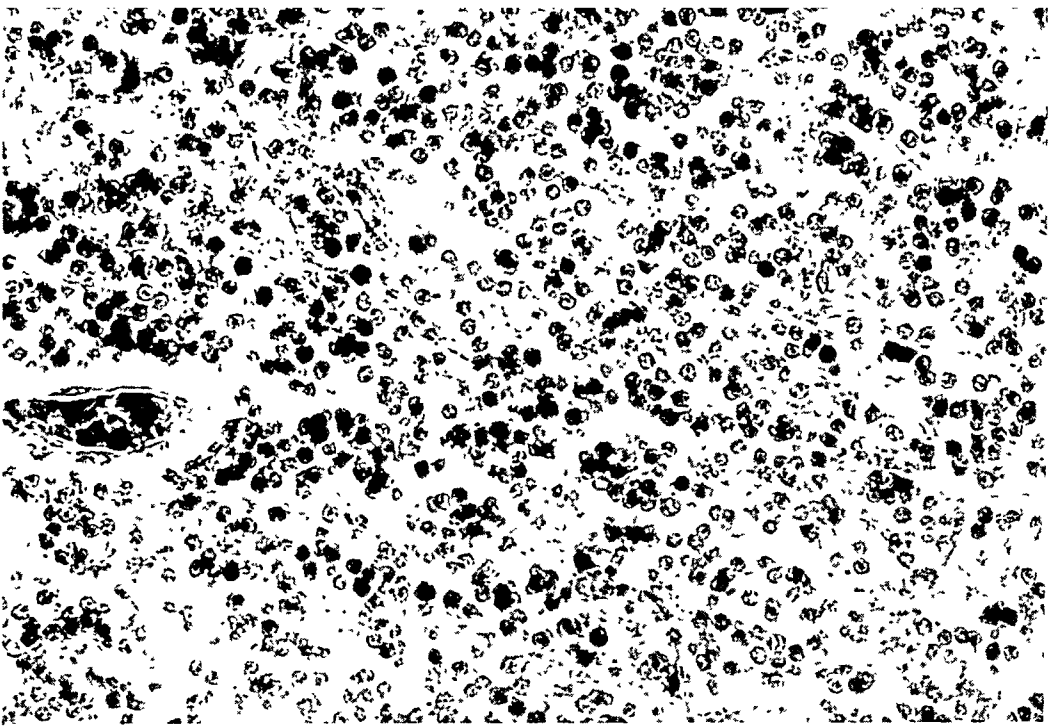


Fig. 9.—Rarefaction of the granular cells and intercellular gliosis in a case of Schilder's disease (diffuse sclerosis). Hematoxylin and eosin stain.

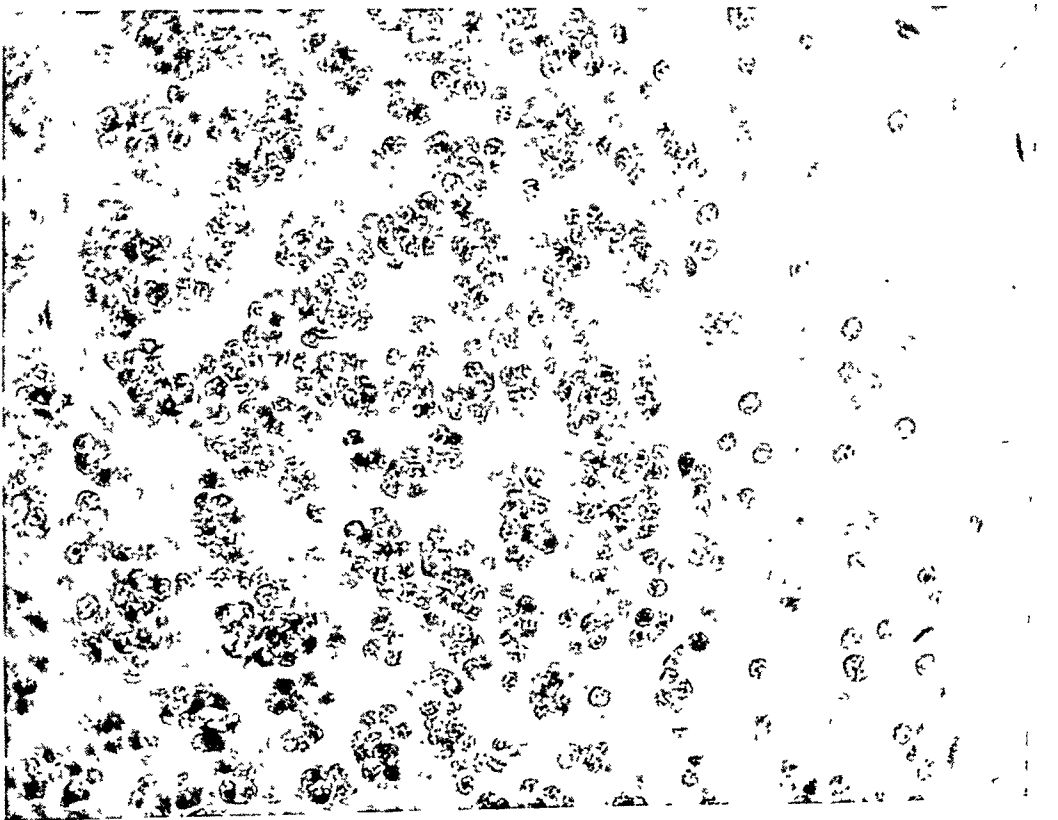


Fig. 10.—Swelling of the cells of the granular layer in a case of electrocution. Hematoxylin and eosin stain.

gradually undergo degeneration. Such a condition has been termed by Ferraro and Morrison *conglutination of the granular cells*.

Conglutination of the granular cells is not a specific process, but occurs under a variety of pathologic conditions. It is found in acute as well as in chronic processes, but seems to be more pronounced in toxic and infectious conditions.

Besides the process of conglutination other changes are described:

1. Rarefaction of the granular cells, a condition which might be the result of a previous conglutination. The rarefaction of the granular cells can, however, be independent of conglutination.

2. A process of acute swelling of the granular cells in which the single elements appear swollen, at times vacuolated, with a tendency to become more distinct and, apparently because of the hydropic condition, to stain poorly. This dropsical condition has been found following the injection of hypotonic solution in rabbits and in two cases of electrocution.

3. Rarefaction of the granular cells associated with interstitial gliosis was found in two cases of Schilder's disease.



# General Review

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## SIGNIFICANCE OF SULPHYDRYL AS A GROWTH FACTOR

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PHILADELPHIA

Since the demonstration of glutathione in living material by Hopkins in 1921 numerous workers have studied the rôle which glutathione and sulphydryl compounds in general play in the activities of cells. Among the claims made in this respect, those connecting sulphydryl ( $\text{—SH}$ ) with the multiplication or with the destruction of cells are especially interesting from the standpoint of research on cancer. The evidence brought forward by the various investigators is, however, often inconsistent with well established facts in regard to conditions controlling cell proliferation, and the conclusions drawn by them concerning the significance of sulphydryl for this process are contradictory. A review and a critical analysis of the experimental work on this subject seem, therefore, to be indicated. It is hoped that a study of the evidence presented and a testing of the validity of the conclusions reached may clarify the situation.

### SULPHYDRYL COMPOUNDS AS ESSENTIAL NUTRITIVE SUBSTANCES

It has been known for some time that cystine, the oxidized form ( $\text{S}=\text{S}$ ) of the sulphydryl-containing amino-acid cysteine belongs to that restricted group of amino-acids which are indispensable for normal growth and development of the animal organism and for synthesis of its proteins. Animals kept on a diet deficient in cystine show lowered vitality, greatly retarded growth and defective formation of hair as well as a drop in the glutathione content of the organism (Lightbody and Lewis; Mitchell and Hamilton; Marenzi and Laclau). Gudernatsch and Hoffman showed that the growth of tadpoles kept in a medium containing amino-acids as the sole supply of nitrogen is stimulated by cystine.

Baker demonstrated that the growth of sarcomatous fibroblasts explanted into an artificial, nutritionally deficient medium was markedly stimulated by the addition to the medium of glutathione in combination with liver ash and hemoglobin. This action on cell growth became apparent, however, only when the cells were grown in a medium deficient

in the proper food material. If fibroblasts were explanted in a medium composed of equal parts of chicken plasma and embryo extract diluted with Tyrode's solution in the proportion 1:25, a moderate growth resulted, which could be appreciably increased if glutathione was added to the medium in amounts equivalent to the amounts present in embryo extract diluted with Tyrode's solution in the proportion 1:4 (Hueper and Russell).

The great prominence which sulphydryl compounds (cysteine, reduced glutathione, thioneine and possibly also thiolactic acid and thioglycollic acid, besides the thioproteins) have obtained in recent years is, however, due not to their nutritive value but to their possible importance as cellular catalysts which control and regulate, directly or indirectly, vital processes of the cells, including especially cell multiplication and cell destruction by proteolysis.

#### SULPHYDRYL AND NORMAL GROWTH

Among the authors who have stated that the sulphydryl group is essential for cell multiplication and acts as a stimulus to this process, Hammett has made the most far-reaching claims, and his work may therefore serve as the basis of the present discussion. From the results of his numerous experiments with plant and animal material, working with minute amounts of sulphur compounds, he concluded that sulphydryl, as the substance occurring naturally in the cell, is the essential and universal stimulus to growth by increase in the number of cells, and that the reaction-product derivatives of sulphydryl are the natural inhibitors of this process, so that the stimulation by sulphydryl is normally limited by the inhibitory action of the suboxidized sulphur forms of sulphydryl ( $-\text{SO}$ ;  $-\text{SO}_2$ ;  $-\text{SO}_3$ ) resulting from the normal oxidation of sulphydryl. Cell proliferation is therefore regulated by the intracellular equilibrium of  $-\text{SH}$   $-\text{SO}_3$ , according to Hammett and Reimann. The countless other factors which apparently have an accelerating influence on mitosis exert it, according to Hammett, only through their effect on the sulphydryl groups rather than directly. He asserted, furthermore, that the acceleration and forcing of cell division by the sulphydryl stimulus in cells with a normal chromosomal constitution result secondarily and without direct relation to the action of sulphydryl in an enhancement of differentiation and organization of the cells, which may reach degrees beyond those ordinarily attained. An explanation of the mechanism of the action of sulphydryl in cell division was, however, not offered by Hammett.

In support of his contentions, Hammett pointed out that sulphydryl-containing substances are naturally concentrated in regions where growth by cell proliferation is actively taking place. This observation is correct

so far as it has been shown by various investigators (Lund; White, Baumann and Webster; Camp; Binet, Leon and Magrou) that sulphhydryl-containing substances are accumulated in the young, actively growing parts of plants (root tips and apical ends) while they are diminished or absent in the mature parts. It has also been noted that the breaking of dormancy of potato tubers, gladiolus corms, peas, etc., is accompanied by an increase in reducing substances, especially in glutathione, in the tissues. The increase in the reducing power of the juice of the activated tubers and corms is, however, only one-fourth due to glutathione, the rest of the effect being caused by some unknown reducing substances, among which may possibly be hexuronic acid (ascorbic acid) recently described by Svirbely and Szent-Györgyi as representing vitamin C in plants and having strong reducing qualities.

It may, furthermore, be mentioned that various sulphur compounds, such as thio-urea; sodium thiocyanate, thiosemicarbaside and thioglycolic acid, have been found to be especially effective in breaking dormancy. But there are numerous other agents, such as ethylene chlorhydrine, hydrogen dioxide, hydrogen, nitrogen, acetylene, light and darkness, by which dormant tubers can be activated (Guthrie; Firket and Comhaire). Guthrie stated that chemicals found effective in breaking dormancy are (1) those that produce an increase in the  $p_H$  and hence an increase in sulphhydryl groups and (2) those that produce only small changes in the  $p_H$  and the reducing power of the juice, such as potassium sulphocyanate and thio-urea. However, according to Firket and Comhaire, no relation exists between the intensity of growth and the relative amount of glutathione in germinating peas, and the glutathione is not localized in any predominant manner in the regions of cell multiplication.

That there does not exist any definite rule in regard to sulphhydryl concentration and cell multiplication is also evident from observations of Guthrie, Denny and Miller, who found that the treatment of non-dormant potato tubers with ethylene chlorhydrine resulted, not in an increase, but in a retardation, of the rate of growth in spite of a marked increase in the reducing power of the juice, which indicates a considerable elevation of the sulphhydryl content of the treated tubers above the normal value.

Proliferating animal tissue also apparently contains considerable amounts of sulphhydryl-containing substances, according to the findings of various investigators (Yaoi; Kamiya; Murray; Binet, Leon and Magrou; Voegtlin and his associates). Voegtlin and Thompson stated that the glutathione content of the body declines with age, dropping to about one third of its highest peak in embryonal life, and that this drop runs parallel with that of the growth rate. But though chicken embryo extract contains large amounts of glutathione paralleling those in the

tissues of the body (Yaoi), there is not a gradual decrease in sulphydryl content conforming with the decrease in the growth rate of the embryo and the increase in differentiation. The glutathione content rises from 0.05 mg. per hundred cubic centimeters on the fourth day of incubation to 0.107 mg. on the twelfth day, declining again to 0.058 mg. on the twentieth day.

The growth-promoting effect of embryo extract cannot well be attributed to its content of free sulphydryl-containing substances, or the arrest of unrestricted growth attributed to the accumulation of inhibitory products of the oxidation of sulphydryl in the medium, for Hueper and his co-workers showed that the complete destruction of sulphydryl and the resulting production of oxidation substances in embryo extract by ultraviolet irradiation do not result in a decrease of its proliferation-stimulating qualities. Moreover, the fact that powdered embryo extract (Borger and Zenker) remains potent for more than six months, during which any free sulphydryl is certainly oxidized by the oxygen of the air into substances of the  $-\text{SO}$ ,  $-\text{SO}_2$  and  $-\text{SO}_3$  type (according to Hammett) does not support the assumption of the exclusive rôle of sulphydryl in cell division.

The mere fact that a certain substance is accumulated in proliferating tissue is, moreover, insufficient proof of its relation to cell multiplication. Other substances (copper and arginine, for instance) are present in increased amounts in tissues of this type.

The assumption of Hammett that the sulphydryl content of liver tissue and liver extract is the hematopoiesis-stimulating principle appears to be insecurely founded in view of the fact that the iron and copper present in these substances may also be responsible for the aforementioned action. While Fleming would like to connect the oxidized form of glutathione with the antianemic qualities of the liver extract, Koegel showed that glutathione and other sulphydryl-containing substances accelerate the decomposition of blood *in vivo* on account of the hemoglobin-destroying action of sulphydryl. The presence of an antagonism between sulphydryl and the formation of blood is furthermore suggested by the experiments of Gruhzit with synthetic reduced and oxidized sulphydryl-containing compounds, which when fed to animals produced severe hemolytic anemia. On the basis of these observations it does not seem justified to connect the anemia observed in advanced cases of cancer with a deficiency of sulphydryl in the bone marrow, since no proof of the existence of such a condition has been offered.

This assertion is not supported by the fact that in anemic conditions the glutathione content of the individual erythrocytes is increased (Gabbe; Woodward and Fry; Varela; Nuomarco and Munilla), while the absolute amount of glutathione of the blood is decreased. This

observation agrees well with the finding of a normal or a low normal glutathione content in cancerous blood (Chatain; Varela, Apolo and Vilar; Woodward and Fry; Binet and Arnaudet; Ulibarri). The suggestion of Ulibarri that the low normal values obtained in cancerous conditions are caused by an absorption of sulphydryl by the malignant tissue does not seem to be justified in the light of evidence to be given later.

The presence of increased amounts of copper in organs and tissues rich in glutathione is of special significance, because it has been maintained that copper catalyzes the oxidation of sulphydryl (Locke and Main; Elliot; Harrison) and that heavy metals, such as copper, lead, gold and arsenicals, are detoxicated in the organism by sulphydryl compounds (Voegtlin; Keesex; Labes). This interrelation gains importance for the problem, as Voegtlin, Rapkine and Hammett have used various heavy metals in their experiments to prove the thesis that sulphydryl stimulates or even is essential to cell division. Rapkine, using sea-urchins' eggs, obtained an inhibition of mitosis by the addition of mercuric chloride to the culture medium. Voegtlin, adding minute amounts of copper to the saline medium containing glutathione in which amebas were suspended, also found a decrease in the number of mitoses. Lead was used by Hammett in his experiments on root tips and paramecia; small amounts of lead salts added to the culture medium always caused inhibition of cell division. Hammett noted, furthermore, that no glutathione could be extracted from the root tips after the treatment with lead salts. He mentioned that the lead did not interfere with the growth in cell size and that extranuclear deposition of lead occurred most markedly in the regions where the concentration of glutathione was the greatest.

The determination of the lead content of the organs of animals that have received lead treatment does not support the view that lead is preferably deposited in the organs containing the highest amount of glutathione. Lead is found in largest amounts in bone, and then, in order, in muscle, kidney, liver and nerve tissue, while no appreciable quantities of lead are deposited in the skin (Aub, Fairhill, Minot and Reznikoff). If the organs mentioned were classified according to their glutathione content, they would appear almost in reverse order. It is known, furthermore, that the binding of the sulphydryl groups in the skin by arsenic (Voegtlin) results not in inhibition of growth, but rather in stimulation of cell growth, as hyperkeratoses and even carcinoma are often seen after prolonged arsenical medication (Hueper and Itami).

The rather discouraging results obtained with the lead treatment in that manifestation of growth *par excellence*, the cancer, do not point to a special affinity of proliferating, sulphydryl-containing tissues for

lead. As it has been maintained by Voegtlin that an increased glutathione content of the tissues protects against the toxic effects of heavy metals, one would suppose that not the proliferating cells with their higher sulphhydryl content (Shearer; Chatton, Lwoff and Rapkine) but the resting cells would be most susceptible to the toxic action of heavy metals.

Heavy metals have, doubtless, affinities other than those to the sulphhydryl compounds. Eichholtz listed as heavy metal complex-formers hexophosphoric acid, histidine, lecithin and other lipoids, pyrocatechine and guanidine derivatives and fragments of the hemoglobin molecule. Toxic action on cells by heavy metals can therefore be brought about not only by an elimination of the undoubtedly important sulphhydryl groups but by interference with other substances essential for the proper functioning of the cellular processes, especially enzymatic activity, which might secondarily result in an inhibition of the reproductive process.

Hammett maintained that the inhibition of growth seen after the exposure of tissues to weak, nondestructive doses of radiant energy is due to the destruction of the sulphhydryl compounds in the cells. The contention is based on his observation of inhibition of growth and retardation of differentiation in marine eggs irradiated for from five to ten minutes with gamma rays from a 15 mg. radium element, and on the decrease in color of the nitroprusside reaction in a watery solution of glutathione exposed for from six to twenty-four hours to the same dose of radium. Hammett was supported in this supposition by Coldwater, who irradiated planarians with 2,000 roentgens and saw simultaneously with the disappearance of the glutathione in the organisms a drop in their mitotic index from 83 to 65.75. In contrast to the minute amounts of radiant energy used by Hammett, Coldwater employed definitely destructive doses. He showed in subsequent experiments that the growth-inhibiting effect of the x-rays could not be neutralized by the addition of sulphhydryl.

From the investigations of Hueper and his co-workers on embryo extract irradiated with large doses of beta and gamma rays, x-rays and ultraviolet rays, it appears to be unlikely that the rays used by Hammett and Coldwater exert an appreciable and direct effect on the glutathione content of the cells. Using embryo extract, a biologic material similar in many respects to the protoplasm of the cells and possessing eminent growth-stimulating qualities, Hueper and his co-workers irradiated this substance with from 15,600 to 31,200 roentgens and 255 and 323 millicurie hours. The results were as follows: X-rays have no destructive action, but possibly slight oxidation occurs: beta and gamma rays cause partial oxidation of the glutathione and slight destruction, that is, oxidation beyond the oxidized form ( $S = S$ ). The same effects were produced

when glutathione in watery solution was exposed to the same types of radiant energy. Ultraviolet irradiation, on the other hand, caused marked oxidation and destruction of the glutathione. Glutathione in its reduced as well as in its oxidized form had entirely disappeared from the embryo extract in some instances after eight hours' exposure to ultraviolet rays.

These results prove rather definitely that x-rays and gamma rays used in therapeutic doses, especially in weak, nondestructive amounts, cannot have any appreciable direct oxidizing effect on the glutathione content of the cell, and that therefore the inhibition of growth observed after the application of these agents cannot be due to the direct elimination of the sulphydryl and the production of suboxidized sulphur in the cells. If the sulphydryl reaction becomes negative or decreases in intensity in the irradiated cells, this must be secondary and attributable to some primary changes in the protoplasm, for instance, in the enzymes (Hueper), caused by the rays.

Other observations also indicate that it is unlikely that radiant energy in small doses exerts a temporary inhibitory effect on cell growth. The carcinoma resulting from repeated prolonged exposures to small amounts of x-rays is well known. If the intracellular sulphydryl groups are so readily destroyed, as apparently Hammett and Coldwater assume, the existence of tumors which are refractory to roentgen rays and radium becomes unexplainable. Moreover, the occurrence of osteogenic sarcomas on the basis of chronic radium poisoning (Martland), a condition in which the cancer-producing tissues are constantly exposed to bombardment with beta and gamma rays, cannot be reconciled with the aforementioned claim. But even the application of ultraviolet rays, which, as has been shown by Hueper and his co-workers, Lieben and Molnar, and others, are able to produce oxidation of sulphydryl, does not result in inhibition of growth but can be the cause of malignant proliferation of cells, as it has been demonstrated by Putschar and Holtz, Herlitz, Jundell and Wahlgree, Abrikosoff and Weil, and Findley and Lawrence that prolonged exposure to ultraviolet rays can produce cutaneous cancer in man and in animals.

To add further support to the sulphydryl theory, Hammett and his co-workers performed experiments in which minute amounts of various sulphydryl compounds were mixed with the culture medium or injected into the organisms. An increase in the number of mitoses was noted in the growing root tips of Zea rays and in cultures of *Paramecium* when *p*-thiocresol or hydrogen sulphide was added to the mediums. The increase in proliferative activity was accompanied by a decrease in cell size. In the hermit crab acceleration of regenerative processes was observed after the addition of *p*-thiocresol to the sea

water in which the eggs were kept. Similar investigations in regard to cell proliferation were made on other marine eggs. *p*-thiocresol and glutathione, respectively, were added in similar concentrations to the sea water, and not only an acceleration of cell multiplication but an enhancement of cell differentiation and organization resulted in the experimental animals. The intratumoral injection of small amounts of thiodextrose caused, according to these authors, a more rapid growth of the tumors in comparison with that seen in tumors which received only equivalent amounts of dextrose. The local application of a 5 per cent benzyl mercaptan ointment to the skin of mice for from four to six months caused on the larger part of the body a thickening of the epithelium through proliferation of the younger, incompletely differentiated cells with the development of a regular base line, spinous cells and keratin and a downward growth of epithelial strands into the corium. Even an increase in the number of hair follicles was noted. In the remaining areas of the body, the epithelial changes were less pronounced: there was thickening of the connective tissue with an increase in vascularization and in elastic tissue formation. An inflammatory reaction was not present, according to the statements of Hammett and Reimann. The two authors reported also a hastening of the epithelization of cutaneous ulcers and a thickening of the epithelium of freshly healed wounds after application of a *p*-thiocresol ointment.

Some of the experiments mentioned have been repeated by other investigators, with negative results. Gaunt, using the eggs of two types of fresh water snails, added cysteine to the water and found that there was no acceleration of cell division as compared with eggs kept in plain water, while, following Hammett's example and advice, there was some increase in the number of mitoses in the cysteine series if equivalent amounts of *d l*-alanine were added to the water of the control series. The conclusion was therefore drawn that there was no evidence of a growth-stimulating action of cysteine and that the increase noted in the last mentioned experiment was spurious, since cysteine in the concentrations used was less toxic than *d l*-alanine.

Similar experiments were performed by Morgulis and Green with *Podarke obscura*. These authors added *p*-thiocresol, thiophenol, thioglycollic acid and cystine to sea water in concentrations approximating those employed by Hammett. No growth-stimulating effect was observed if the sulphhydryl-containing substances were used in concentrations of the "effective" range, according to Hammett, while cell degenerations occurred when concentrations above this range were used, a result which to a certain extent was parallel to that obtained by Sullivan on feeding excessive amounts of cysteine to animals. Morgulis and Green objected, moreover, to the method of calculation used by Ham-



mett in the evaluation of his results, which tends to exaggerate grossly the actual differences.

Attention may be called in this connection to a statement of Lewis, that thiophenol and thiocresol resist oxidation in the organism as all mercaptan groups directly attached to a benzene nucleus do, while sulphur linkages of the character of the mercaptan group, or those which can easily be hydrolyzed to form this group (thio-urea or cysteine), are easily oxidized. The sulphydryl group in *p*-thiocresol is therefore in the organism rather stable and consequently chemically inert. For this reason, it is probable that the sulphydryl group in glutathione and in *p*-thiocresol will have a different biologic effect.

It is also unlikely that the hastening of healing of wounds by the application of extracts from macerated tissue can be attributed to the effect of sulphydryl as noted by Hammett, who referred to the observations of Carnot and Terris on extracts of macerated skin. Hammett's claim is based on the statement of Bierich and Kalle that, in autolysis, a liberation of sulphydryl-containing substances occurs; these, according to him, act as growth stimulators. McJunkin repeated the experiments of Carnot and Terris and found that macerated skin apparently accelerated the healing of cutaneous wounds, but that, on the other hand, the application of extracts of liver, which, as is well known, contains large amounts of glutathione, did not favor healing, but rather inhibited it. The observations made during experiments *in vivo* have been substantiated by experiments *in vitro*. Fischer mentioned that liver extract is the only extract which definitely inhibits cell proliferation. This finding was substantiated in the experiments of Hueper and his co-workers with normal and malignant tissues *in vitro*. Moreover, observations on tissue cultures do not support the assumption that the liberation of sulphydryl in cell degeneration stimulates proliferation because partial necrosis in tissue cultures inhibits growth (Fischer).

In reference to Hammett's experiments on marine organisms with sea water as the medium, it may be pointed out that some of the marine organisms contain, according to Blanchetière and Melon, not inconsiderable amounts of glutathione (from 24 to 174 mg. per hundred grams of tissue). In view of these relatively high amounts of glutathione in the adult animals and the probability that they are even greater in the eggs and embryos, it seems rather doubtful that the minute amounts of sulphydryl-containing substances added to the sea water should exert any appreciable biologic effect on their growth and development. As additional complicating factors the active iron and other heavy metals, such as copper, gold and manganese, dissolved in the seawater and the rather alkaline reaction ( $p_H$  8 to 8.2) of the medium must also be given consideration. Both of these factors tend to favor

the rapid oxidation of any sulphhydryl-containing substances added to the water (Harvey). It seems, therefore, to be rather improbable that the sulphhydryl that was added reached the experimental animals in an unchanged condition.

Concerning the use of hydrogen sulphide as a substance containing sulphhydryl and therefore stimulating mitosis (Hammett; Sharpe), Sun did not observe such an effect in sea-urchins' eggs. It cannot be assumed as certain that hydrogen sulphide and glutathione exert the same effect on the cell, as there is apparently a difference between the action of hydrogen sulphide and that of reduced glutathione on the respiratory mechanism (Dixon; Bumm and Appel). Hydrogen sulphide is, moreover, an exquisite cell poison, comparable in this respect with cyanogen (Moncorps).

The interpretation of the epithelial changes produced in the skin of mice painted with benzyl mercaptan as evidence of phylogenetic progression caused by sulphhydryl seems to be rather far-fetched in view of the fact that similar formations can be produced in the mouse by other means (tar, 1:2:5:6 dibenzanthracene) and are common among the pathologic changes of human mucous membranes (leukoplakias). The pictures published with the respective papers do not support, moreover, the contention that the epithelial proliferations occur in the absence of an inflammatory reaction, because several pictures show a moderate but definite small round cell infiltration of the corium. The epithelial changes may, therefore, more likely be due to a low grade chronic inflammation. Considering also the facts that sulphhydryl compounds are apparently used for the synthesis of keratin (Guroud and Bulliard), and that this process tends to be initiated in surface epithelial cells when through increase in the number of cell layers a gradual removal of the upper layers from the base of food supply occurs and thereby an interference with nutrition takes place (Mekie), the rôle which sulphhydryl plays in phylogenesis does not appear to be as definitely established as Hammett asserts. The toxic nature of the sulphur compounds used in these experiments is well demonstrated by the occurrence of severe cutaneous reactions with formation of blisters in some instances in which 20 per cent thiocresol ointment was used (Reimann).

Moreover, the work of Voegtlin and his co-workers cannot be regarded as proof of the promotion of cell division by sulphhydryl. Using amebas as test objects and reduced and oxidized glutathione in a buffered saline solution as the sulphhydryl-containing medium, Voegtlin observed an increase in the number of mitoses in the experimental series in comparison with the control series which were kept either in the plain buffered saline solution or in a saline solution to which equivalent amounts of several amino-acids that do not contain sulphhydryl were

added. It was noted that the addition of sulphhydryl to the medium hastened the digestion of the material in the food vacuoles. The stimulation of cell division depended on the size of the cells. The small cells appeared to be refractory to stimulation with sulphhydryl, indicating that the effect was a function of cell volume. Considering cell volume as a function of physiologic maturity or "differentiation" of the cell, the effect of glutathione depends, according to Voegtlin, on this factor. These observations and conclusions stand partly in direct contrast to those of Hammett, who connected the susceptibility to the sulphhydryl-stimulus with the immature, small cells.

Certain objections can be raised against the experiments described. Among the amino-acids added in equivalent amounts to the control medium was alanine, which was also used by Hammett and Gaunt in their controls. As already mentioned in connection with Gaunt's work, alanine proved to be cell-toxic. That a similar action was exerted in Voegtlin's experiments is apparent from the survival rates of amebas kept in three different mediums (with glutathione, about 70 per cent; with plain saline solution, 60 per cent, and with saline solution plus alanine, 55 per cent). The amebas in the alanine-containing medium showed the lowest survival rate. It is obvious that such a toxic effect must have a definite influence on the rate of cellular division. No consideration, moreover, has been given to the possibility that glutathione might have served as an activator of proteolytic enzymes engaged in the degradation of the protein material contained in the dead amebas, thereby supplying the living organisms with certain quantities of food substances and in this way supporting their survival and proliferation. That such a process may have been active seems to be indicated from the observation recorded that the rate of digestion of the material in the food vacuoles of the amebas in the glutathione medium was accelerated.

In the selection of amino-acids for this type of experimentation, consideration must be given to the fact that the various amino-acids have different effects on the maintenance, growth and differentiation of the organisms used (Gudernatsch and Hoffman) or reliable and conclusive results will not be obtained. On account of the aforementioned conditions, Voegtlin's observations remain of doubtful value so far as the stimulating action of sulphhydryl compounds on cell division is concerned.

The amount of intracellular sulphhydryl evidently depends to a certain extent on the availability of oxygen, being increased whenever there is an insufficiency of oxygen. This deficiency of oxygen may be absolute or relative. When an absolute deficiency prevails a condition more or less incompatible with life exists and degenerative changes result. Owing to the decreased respiratory activity of the cell, an emergency and

auxiliary fermentative metabolism is resorted to by the cell for the production of the energy needed for the continuation of the vital processes. The lactic acid thus generated shifts the reaction of the cell toward the acid side, thereby favoring the stability and the production of the reduced form of sulphhydryl in the cell. This type of intracellular increase in sulphhydryl may be designated as the degenerative form.

On the other hand, excessively increased oxidative processes in the cell which demand an oxygen supply surpassing that which can be met by the normal resources of the tissue may result in a temporary and relative deficiency of oxygen and thereby cause an increase in cellular sulphhydryl. This hyperoxidative type of intracellular increase in sulphhydryl is apparently present in hyperthyroidism (Handovsky) and in organs with active cellular proliferation. It is obvious from these considerations that the presence of an increased amount of sulphhydryl in a certain tissue may characterize two biologically different cellular states.

The evidence presented by the investigators cited in the foregoing discussion on the growth-promoting qualities of sulphhydryl-containing substances does not offer sufficient proof of the thesis that sulphhydryl is the essential and universal stimulus for normal growth in plants and animals. It remains doubtful whether the biologic activity of sulphhydryl compounds favors cell division under all circumstances, or at all, as there exists evidence, partly already given and partly to be discussed later, which indicates that they may possibly act also as growth inhibitors.

#### SULPHYDRYL AND CANCEROUS GROWTH

In conformity with the claims made concerning the significance of sulphhydryl compounds in regard to normal cell growth, several authors have attempted to relate these substances to the development and the proliferative activity of malignant tumors.

Hirsch asserted in his glutathione theory of canceration, as reported by Delbet and Franicevic, that canceration occurs when the glutathione accumulates or is at least relatively increased in the tissue, thereby disturbing the normal balance between the glutathione in the tissue and that in the blood. He bases his contention on the following observations: The glutathione content is elevated in the tissues of young animals and lowered in the blood during the period of greatest growth; in adult animals, chronic irritation causes an increase of sulphhydryl in tissue comparable to that present during growth and thus produces a carcinogenic condition in the tissues; tar and arsenic lower the amount of glutathione in the blood, creating in regard to tissue glutathione a similar condition, that is, a relative increase and consequently a change in the oxidation-reduction potential between the tissues and the blood, which,

according to Hirsch, determines cell growth; young tumors contain an increased amount of glutathione, while it is diminished in the blood, and finally acidosis increases the glutathione in the blood, thereby removing a condition favorable to the development of cancer and cell multiplication in general.

Chatain is another investigator who has maintained that glutathione is a factor favorable to cancerous growth; he pointed out that this substance is increased in malignant tissue, and that cancerous growth is stimulated by insulin owing to its labile sulphur group.

Extending his conception of the action of sulphydryl on normal cell proliferation to the causative mechanism of malignant growths, Hammett proposed the following theory of the origin of cancer, which has been adopted and elaborated by Reimann: The biologic basis of malignant tumors is the distortion of the sulphydryl equilibrium in the presence in the body of genetically determined lines of cells in which the heightened nuclear reactivity of hypersensitivity to stimulation to proliferation by sulphydryl characteristic of young, incompletely differentiated cells is retained without resulting secondarily in increased differentiation. As the diverse architecture of malignant growths is essentially a consequence of the diverse anatomic and physiologic environments in which the proliferation of cells takes place, and as the increase in mitotic activity is in general followed by a decrease in the size of the cells, the rate of tumor growth is directly related to the interpretation of malignancy in terms of sulphydryl calculated from cell size. Sarcomas and carcinomas represent different responses to stimulation with sulphydryl in persons of different constitutional (connective tissue or epithelial) types. The difference in hydrogen ion concentration, carbohydrate metabolism and proteolytic activity of the malignant cell in comparison with the normal cell are but sequelae of the heightened reproductivity of the malignant cell.

In support of this theory, Hammett referred to the statement of Voegtlin and Thompson that tumor tissue contains glutathione in amounts comparable with those present in the liver, the organ having one of the greatest quantities of glutathione present in the various tissues. This high concentration is interpreted as due to the fact that tumors are composed of proliferating cells. The high sensitivity of embryonic cells to the sulphydryl stimulus is claimed to account for the development of tumors from embryonic rests (Cohnheim). The increased reduction power of cancerous blood, reported by Roffo, was attributed by Hammett to the probable presence of an increased amount of sulphydryl in the blood resulting from an overproduction of this substance by the malignant tissue. In reference to Roffo and Corea's observation in regard to the existence of insulinoid substances in tumor tissue, it is pointed out that, according to the observations of Adlersberg and

Perutz, insulin, containing suboxidized sulphur, stimulates the healing of old ulcers of the leg—a growth-stimulating effect which Hammett, however, could not substantiate in experiments with plant roots and paramecia. The observation that the loss of activity of Rous' sarcoma filtrate can be delayed by the addition of cysteine or glutathione (Gye and Purdy; Muller) is taken as indication either that the active growth principle present in this filtrate is sulphhydryl or that its effectiveness is in some way related to sulphhydryl.

The increased consumption of oxygen by malignant tissue, as reported by Russel and Gye, is considered as evidence for the increased concentration of sulphhydryl in cancerous tissue requiring a heightened demand for oxygen. Tar cancer is attributed to the presence and growth-stimulating action of mercaptans and sulphides in the tar, promoting the generative processes beyond the level of repair. The frequency of metastases to lymph nodes is assumedly due to the facts that these organs are foci of cell proliferation and that proliferating cells respond best to the sulphhydryl stimulus, while the high incidence of metastases to the liver is ascribed to the high concentration of glutathione in this organ, which is also supposed to be responsible for the hematopoiesis-stimulating effect of liver extracts, a contention already dealt with. The anemia so often encountered in the advanced stages of malignant tumors is assigned to a decline of the bone marrow, the decline being due to an accumulation of sulphhydryl in the tumor corresponding to a general decrease in the rest of the organs (Voegtlin).

It is furthermore asserted that the hydrogen sulphide produced in the intestines by bacterial action, especially in the presence of constipation, may act as an etiologic factor in the development of cancer in constitutionally inclined persons. The infrequency of malignant growth in persons with hyperthyroidism is explained by the fact that this condition increases the oxidative processes in the body, causing oxidation of the sulphhydryl group, which is highly sensitive to oxidizing agents. The cancer caused by *Spiroptera neoplastica* in rats (Fibiger) is laid to the production and fertilization of eggs, resulting in an increased concentration of sulphhydryl (Shearer) in the tissues adequate to stimulate an abnormal proliferation of cells in a ground prepared by the inflammatory process and constitutionally inclined to a pathologic response.

Saunders held that the changes in the hydrogen ion concentration and the oxidation-reduction potential occurring in chronically inflamed tissues enable the sulphhydryl groups present in the tissues, such as cysteine and glutathione, to act as a stimulus for cell division and to contribute to the causation of malignant growth. Saunders pointed out that, according to Hammett, sulphhydryl is stable at  $p_H$  5.5 and stimulative to growth; at  $p_H$  7.2 it is changed to a suboxidized state, and therefore

retardation of cell multiplication results, whereas at  $p_H$  6 to 7, a range in which an equilibrium exists between the reduced and the suboxidized form of sulphydryl, no consistent response is registered. He expresses the belief that the lactic acid produced by streptococci isolated from inflammatory and neoplastic tissues reduces the  $p_H$  of the tissue to about from 4.2 to 4.8, which keeps the sulphydryl in the stimulating phase.

If the evidence cited in support of the various theories respecting the part played by glutathione in canceration is evaluated, it soon becomes obvious that some of the data either allow a different interpretation or are contradicted by findings of other authors.

The statement of Voegtlin and Thompson that the high glutathione content of malignant tumors is comparable to that of the liver, one of the organs richest in this substance, is incorrect if judged by the figures given by these authors. In no instance does the glutathione content of the tumor (carcinoma and sarcoma) even approach that present in the liver of the animal; usually it is far below it, often being only one half or one third of that of the liver, or less. Bierich and Kalle stated, for instance, that malignant tissue contains approximately the same amount as normal tissue, adding that the glutathione content of a tumor apparently depends on its cellularity. A similar opinion was expressed by Hieger and Kennaway. Binet, Leon and Magrou noted that a greater amount of glutathione is present in normal growing tissue than in cancerous tissue. Holmes as well as Heinlein reported abnormally small quantities of glutathione in malignant tumors. Yaoi and Nakahara also recorded the presence of negligible amounts of glutathione in Rous' tumors, making Hammett's assumption concerning the relation of sulphydryl to the active principle of this tumor rather unlikely, especially in view of the observation of Murphy, who found that the nitroprusside reaction is negative in purified Rous' filtrate.

Voegtlin and Thompson observed a decrease in the glutathione content of the organs with the progress of the neoplastic growth. This observation was in general substantiated by Medvedev, who stated that carcinomatous tissue has a low glutathione content, but it was not confirmed by Bierich and Kalle. It must, however, be mentioned that apparently there is a drop not only in the glutathione content of the normal tissue, but in that of the tumor, according to the figures of Voegtlin and Thompson and Medvedev. These observations throw an interesting light on the investigations of Saxl, Kimura, Kahn and Postmontier and of Moravek on the total sulphur content of the urine and blood in cancerous persons. It was found by these workers that the total sulphur is increased in the urine and blood and decreased in the tumor tissue. These findings and the fact that various carcinogenic agents, such as arsenic, aniline and tar, are detoxicated in the body by sulphur (sulphydryl, according to Voegtlin and others) have caused

Grumme and Medvedev to maintain that cancer is a disease based on a deficiency in sulphur.

The question in regard to the state in which glutathione is present in the tumor tissue is still controversial. While Voegtlin and Thompson asserted that the glutathione in the tumor occurs mainly in the oxidized form, Bierich and Kalle expressed the reverse opinion.

As the majority of the authors cannot establish any extraordinary abundance of glutathione in neoplastic tissue or any relation between the amount of sulphhydryl and the malignancy of the tumor (Binet, Leon and Magrou), no conclusions of definite value in regard to the significance of sulphhydryl in the genesis and development of malignant neoplasms can be drawn.

The theory that glutathione is concerned in metastasis (Hammett) does not take account of the fact that mechanical factors regulate to a marked extent the distribution and implantation of secondary growths in the lymph nodes and the liver. The frequency of metastases in the lung, an organ with a moderate glutathione content and an excellent oxygen supply, remains entirely unexplained by such a theory. Primary tumors in the liver are, moreover, relatively uncommon, and metastases in the spleen, a lymphoid organ, are rare.

The anticancerous action of hyperthyroidism, referred to by Hammett as due to the stimulation of the oxidative processes in the body and the resulting destruction of sulphhydryl, can also not be attributed to this mechanism, as Handovsky found that the feeding of thyroid tissue causes an increase of the organic glutathione. Handovsky added that whenever that tissue has an insufficient supply of oxygen either on account of poisoning of the respiratory ferment or on account of increased oxidation, glutathione in the tissue is increased. It must, furthermore, be kept in mind that glutathione as a respiratory catalyst accounts for only a part of the tissue respiration (Mann), and that cancer cells obtain their main source of energy from glycolysis, a process which cannot be suppressed by an increase of oxygen pressure, as the interrelation between respiration and fermentation expressed in the Pasteur reaction for normal cell metabolism does not exist for malignant cells.

It seems to be doubtful, moreover, that the acid reaction present in inflammatory tissue acts as a growth stimulant through its effect on the equilibrium between reduced and oxidized sulphhydryl, causing a shift toward the reduced sulphhydryl side. It is rather well established that normal as well as malignant cell proliferation is inhibited at an acid hydrogen-ion concentration ( $p_H$  5.5), which is regarded by Hammett as the optimum for the stimulating effect of sulphhydryl, and that, on the contrary, the growth optimum is found in the alkaline range at about



$p_H$  7.4 to 8 (Fischer; Balint and Weiss; Marton and Magassy, and many others), where sulphhydryl is considerably less stable.

Demuth remarked that the growth of tumor cells proceeds less rapidly with an increase in the amount of lactic acid produced, and Reiss pointed out that the depression of the  $p_H$  of the environmental medium of malignant cells by the accumulation of lactic acid, either as the result of an excessive production of this substance or as the result of interference with its removal, may work as an inhibitory mechanism of self-regulation of tumor growth.

Considering the natural tendency of tissues to reduce glutathione (Dixon; Schiff and Fukuyama) and the fact that reduced glutathione becomes more stable and therefore inert (Mann), with an acid reaction (Joyet-Lavergne), and knowing that cell mitosis is a process requiring large amounts of energy (Needham) which are probably derived from oxidative processes and not from reducing ones, as Rapkine implied, one cannot readily conceive how a sulphhydryl group of great stability, such as that present in acid tissue, could be responsible for the induction of mitosis. On the other hand, the great lability of the equilibrium between reduced and oxidized glutathione (SH—SS) in a biologic medium of alkaline reaction in which the reducing quality of the tissue is counteracted by the oxidizing tendency of the hydrogen ion concentration indicates that an SH—SS constellation of great reactivity should exist which might well take care of or participate in the production of the energy needed in mitosis and protein synthesis without which continuous cell proliferation is not possible. Hopkins attested to the higher reactivity of sulphhydryl in an alkaline medium than in an acid one when he observed that oxidized glutathione is not an autoxidator in an acid medium, while it is in an alkaline one, where it is constantly reduced by the hydrogen donors in the presence of dehydrogenases of the tissue, as the autoxidation optimum of glutathione is found at  $p_H$  7.4 (Dixon and Tunncliffe). On the other hand, Hopkins demonstrated that on the acid side of  $p_H$  7.4, the protein sulphhydryl is oxidized, and the total amounts to ten times the equivalent of the sulphhydryl present, while at  $p_H$  7.4 to 7.6, the oxygen uptake amounts to only sufficient to oxidize sulphhydryl.

The optimum hydrogen-ion concentration for growth is, moreover, closely related to the optimum hydrogen-ion concentration for glycolysis, which is found at  $p_H$  7.58; glycolysis is almost lacking at  $p_H$  5.8, according to Reding, Warburg and others. The restraining effect of an acid reaction on malignant growth, in spite of an apparently favorable concentration of sulphhydryl, is therefore readily explained by the resulting inhibition of glycolysis, the main source of energy of the cells (Warburg, Posener and Negelein). The proper functioning of this important metabolic mechanism depends apparently to some extent on the alkalinity

of the medium. Sugar and sulphydryl compounds, being ubiquitous systems (Kuehnau), are evidently intimately interrelated in their biologic activity, as a high glutathione content is found in organs and regions which are centers of sugar metabolism (liver, suprarenal glands) (Kuehnau), a finding confirmed by Joyet-Lavergne. Niethammer observed, moreover, that germination of seeds does not occur and cannot be forced in the absence of sugar.

The direct dependence of the sulphydryl concentration on the intensity of the sugar oxidation is also shown by the fact that under certain conditions (dextrose oxidation) the liver can rapidly form large amounts of glutathione, from unknown sources, as long as the hepatic function is intact, while the sulphydryl fraction drops and becomes zero when this process is arrested.

The existing interrelation between the hydrogen ion concentration of the medium and the biologic activity of the sulphydryl system in malignant growth becomes significant in view of the considerable evidence which has been collected on the tendency of cancerous blood to be alkaline. Klobliha noted that proliferating cells have a somewhat less alkaline cytoplasm than normal cells, but that the cytoplasm of malignant cells, even if it is not basic by its own resources, can become alkaline by the action of the alkaline blood. Recent investigations with cultures of malignant cells kept under aerobic conditions have, moreover, shown that the supernatant fluid and the plasma-embryo extract medium become increasingly alkaline instead of increasingly acid as in anaerobic cultures (Hueper and Russell). As the aerobic conditions in the alkaline cultures correspond more closely to those present in the peripheral parts of malignant tumors, where the actively proliferating cells are in direct contact with the vascular system of the invaded normal tissues and therefore obtain an approximately normal supply of oxygen, these results may throw a new light on the vital activities of malignant cells *in vivo* and on the factors regulating them.

It is apparent from these data that the conditions existing in tumor tissue are not absolutely favorable to the stability of reduced cellular glutathione, but rather tend to make it labile and susceptible to oxidative agents. A growth-stimulating effect of sulphydryl-containing substances injected in minute amounts into tumors, as maintained by Hammett, is therefore scarcely probable and has not been confirmed by Gilroy.

The results of the great majority of investigators contradict the contention of Chatain and Hammett that insulin stimulates tumor growth (von Witzleben; Stuehlern; Piccaluga and Gioffari; Muenzer and Rupp, and others). It is, *a priori*, unlikely that a substance which decreases the dextrose content of the blood from which the tumor cells obtain their supply of this substance would act stimulatively on cell prolifera-

tion. Insulin and sulphydryl are, moreover, antagonists, as insulin is inactivated by cysteine (duVigneaud).

In regard to Hammett's assumption that the carcinogenic action of tar is due to its content of mercaptans and sulphides, reference may be made to the work of Burrows, Hieger and Kennaway, and Cook on the carcinogenic effect of synthetic benzantracenes which they developed from their study of the purification products of tar, and which they relate to the carcinogenic quality of this substance. As these compounds do not contain any sulphydryl groups, this claim of Hammett seems to be rather insufficiently founded.

From the available evidence, as presented in the foregoing discussion, it can be concluded that the various theories as to the etiologic relation of glutathione and sulphydryl to canceration are not properly supported by definite facts. The great mass of observations and findings rather disproves them than sustains them. If one grants sulphydryl substances a contributory part in the development of malignant growth, it can be at best only a secondary rôle, remaining in the framework of their normal function.

#### ACTIVATION OF CELL DESTRUCTION BY SULPHYDRYL IN CANCER

The theoretical as well as practical weakness of the theories concerning sulphydryl and malignant growth is furthermore emphasized by the conceptions which are held by Waldschmidt-Leitz and Voegtlin in regard to the interrelations existing between the proteolytic processes and the activation of cathepsin by sulphydryl in malignant tumors.

On the basis of the observations of Grassmann that only reduced glutathione is an activator of proteolysis by cathepsin, Waldschmidt-Leitz proposed the theory that a connection exists between the oxidative and the hydrolytic processes in cancerous tissue. He contended that while in the normal cell, on account of the ample oxygen supply, the SH—SS equilibrium is shifted toward the oxidized form of glutathione, in the malignant tissue the existing oxygen deficiency, the production of lactic acid and the resulting acid reaction favor the stability of the reduced form of glutathione, resulting in optimal conditions for the activation of the intracellular protease, cathepsin, by sulphydryl. Waldschmidt-Leitz maintained that this constellation is responsible for the increased cellular proteolysis in malignant tumors, which is still more enhanced by the fact that the activator of cathepsin is considerably increased with tissue decay. In support of his theory, Waldschmidt-Leitz stated that the glutathione content of mature or old tumor tissue is higher than that of young tumor tissue. He referred also to the work of Mothes, who found that the activator of protein hydrolysis in plants is a sulphydryl-containing substance, while that of protein synthesis is

one of the character of oxidized glutathione (GSSG). The direction of protein metabolism in the plant depends, according to this author, on the oxygen potential. If oxygen is present in insufficient amounts, hydrolysis is activated; if it exists in excess, synthesis occurs. From these statements it can be inferred that any increase in sulphhydryl in the cell will cause a tendency to cellular proteolysis or cell decomposition, while a shift in the intracellular SH—SS equilibrium in favor of the oxidized form (SS) will stimulate protein synthesis, a process which appears to be essential for cell division. Whereas protein synthesis and protoplasmic synthesis are not directly synonymous with mitosis, cell multiplication cannot continue for any length of time if this process is not only inhibited, but counteracted, by protein decomposition resulting in cell destruction.

Voegtlin and Mavern expressed similar views in a study of the relation between the oxidation and the proteolysis of malignant tumors. The occurrence of necroses in tumor tissue is explained by them as due to the existence of an inadequate vascular supply and consequently a deficient circulation of blood in parts of the tumor, resulting in insufficient food supply, accumulation of lactic acid and local increase of the hydrogen ion concentration, causing the death of cells. The low oxygen tension and  $p_H$  and the stability of sulphhydryl thus attained stimulate proteolysis by activation of the tissue proteases. This destructive process can, in the opinion of the two authors, extend to the surrounding tissue, while the products of protein degradation are either removed through the blood or utilized by the adjacent tumor cells for growth and multiplication, a process which will simultaneously also be favorably affected by the increase in intracellular sulphhydryl. The conceptions held by Voegtlin and Mavern are essentially identical with those supported by Waldschmidt-Leitz concerning the causative mechanism of tumor necroses and of the destruction of the surrounding normal tissue.

These theories are to a certain extent supported by the observation of Rosenthal and Voegtlin that malignant tissue, the liver and the brain, in contrast with other tissues, keep glutathione for some time in the reduced form. It may also be pointed out that parenchymatous organs which normally have a high glutathione content (liver, suprarenal glands) have a marked tendency to rapid postmortem autolysis. But observations made on isolated dead tissue and on postmortem changes cannot be applied without restrictions to necroses in vivo, which are exposed to the effects of the surrounding living tissue.

Against the validity of these theories numerous objections have been raised. Krebs found that the proteolytic activity of tumor cells is high but within normal limits, while Kleinmann and Werr could not demonstrate in extensive investigations that the catheptic activity of normal

and malignant tissues differs in quantitative or qualitative respects. They concluded, therefore, that no interrelations can be established between increased growth and proteolytic processes in tumors. These results have been substantiated by Malowan and Rondoni. Kleinmann furthermore pointed out that the cathepsin of tumor tissue cannot be activated against its own proteins by the usual method, and that Stern succeeded in this only by means of heavy metal catalysis. This assertion of Kleinmann was, however, disputed in recent investigations of Waldschmidt-Leitz.

Heinlein, contradicting Waldschmidt-Leitz, called attention to the fact that the older portions of the tumor contain not more but less glutathione. Moreover, Morel and Delore and Vosco and Castagna observed that reduced glutathione disappears rapidly from dying and necrotic tissue. Abderhalden noted that the autolysis of minced liver, spleen and kidney is not appreciably influenced by the addition of reduced glutathione, which indicates that proteolysis cannot be stimulated by sulphhydryl beyond a certain optimum. Krebs noted that no definite conclusions as to intracellular processes *in vivo* can be drawn from results obtained with tissue extract, an opinion which was strongly emphasized by Edlbacher also.

The action of sulphhydryl on enzymatic activity *in vivo* and to a certain extent also *in vitro* is not yet sufficiently understood, as it seems to be difficult to distinguish with present methods between a pseudo-activating effect of sulphhydryl on the enzymes by the removal of heavy metal inhibitors (Krebs; Klein and Ziese) and a direct stimulating effect of sulphhydryl (Waldschmidt-Leitz) either alone or in the form of a sulphhydryl-heavy metal complex (Waldschmidt-Leitz; Salaskin and Solowjew). The great complexity existing in this respect, which makes it extremely difficult to obtain a clear understanding of the interrelations of the various factors present in living biologic material, is shown by the fact that the activation of arginase is controlled, not only by a simple interaction of enzyme and activator, but by the synergism and antagonism of amino-acids and heavy metals (iron and copper) and their various types of valencies (Stern and Michaelis); the amino-acids probably act in this complex process as a reducing agent of the metal, or the latter, for instance ferrous iron, as a protector of sulphhydryl from oxidizing agents (Edlbacher). It seems, moreover, to be doubtful if the activation of certain intracellular proteases *in vivo* depends exclusively on the presence of sulphhydryl, as the cells doubtless contain other reducing substances, such as ascorbinic acid, which is accumulated in organs (liver, suprarenal glands) which excel in their glutathione content.

In critical evaluation of these apparently contradictory conceptions and of the evidence presented, attention may be called to the following

points: Necrosis is by no means a constant characteristic of malignant tumors. Proteolytic processes occurring in tumor necroses are evidently secondary to the coagulation of the cell proteins. Rapid proteolysis of necrotic tissue (benign or malignant) is neither constant nor frequent. The majority of infarct necroses after a primary imbibition of the infarcted tissue with extravasated plasma undergo a dehydration process and are slowly lysed in the course of weeks and months by the action of invading leukocytes and phagocytes (Orth). Similar conditions are commonly observed in necrosis of malignant tissue. The proteases liberated from the dead cells and activated by the sulphhydryl compounds are therefore often not able to cause any considerable proteolytic changes in the dead tissue *in vivo*, in contrast to those observed *in vitro* and in postmortem autolysis. This delayed proteolytic degradation *in vivo* may be due to different factors. Sulphydryl substances appearing in necrotic tissue are destroyed by the oxidizing action of the plasmatic material entering the necrotic tissue by exudation from the blood vessels, as it has been shown in recent investigations of Hueper and Russell that plasma oxidizes sulphhydryl compounds added in the course of a few minutes at a temperature of 37 C. Plasma will also tend, on account of its buffer power, to prevent a shift of the reaction of the necrotic tissue from the alkaline to the acid side, which is more favorable to the stability of sulphhydryl compounds and to proteolysis. The blood of tumor carriers has in addition an increased antiproteolytic titer, according to the statement of Wells. There will be, moreover, a drainage of enzymes from the necrotic tissue into the blood owing to the increased permeability of dying and dead cells (Oppenheimer; Mandelbaum). It can also be assumed with some justification that easily diffusible substances, such as the enzymatically important sulphhydryl compounds, particularly those not bound to proteins, are relatively rapidly removed by diffusion from the dead tissue (Morel and Delore; Visco and Castagna).

Vascular disturbances and cell crowding in the tumor, together with the increased need for food material for the maintenance and proliferation of tumor cells, resulting in a keen and certainly sometimes unfavorable competition among the tumor cells for these substances, may account for and contribute to the frequency and exaggeration of necrotic processes in tumor tissues. Similar factors are apparently responsible for the degeneration of the normal tissue exposed to the infiltratively growing malignant cells, accounting for their "destructive" action. This conception in regard to "destructive" growth, which is also applied to many benign manifestations, such as inflammatory hyperplasias and endometriosis, is supported by the observations of Rondoni who noted that tumor cathepsin prepared after the method of Waldschmidt-Leitz shows no special features in comparison with the enzyme of normal

tissues. Price demonstrated that in malignant conditions the surrounding normal tissue is not digested by enzymes liberated from the tumor, and that the growth of the tumor does not depend on the autolytic products resulting from the degeneration of the surrounding normal tissues.

It is evident from these data that the intracellular activation of cathepsin in malignant tissue is not responsible for the causation and frequency of necroses and the infiltrative and destructive growth of malignant neoplasms.<sup>1</sup>

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1. This conception has received additional support by the recent work of Maschmann and Helmert, who noted that the presence of much or especially active cathepsin is not a prerequisite for the orderly growth of embryonic cells or for the disorderly growth of malignant cells and that cathepsin does not participate in the autolysis of malignant cells, as they found almost no cathepsin in necrotic tumor tissue from mice. Borger, Peters and Kurz, who investigated the sulphydryl content of infarcted tissue, pointed out that decrease of respiration and increase of proteolysis do not result in necrosis, as Waldschmidt-Leitz had assumed. They observed that the sulphydryl content of necrotic tissue of artificial and spontaneous infarcts decreases rapidly. One hour after ligation of the vessel a decrease in the sulphydryl content of the affected tissue was found, while after from forty-eight to seventy-two hours only 10 per cent of the original sulphydryl content was left. The decrease of sulphydryl in the infarcted tissue was due to oxidation, a process which was favored by the relatively alkaline reaction of the necrotic tissue ( $p_H$  7.1 to 8.4 against a normal  $p_H$  of 6.4 to 7). They concluded that a marked action of cathepsin in necroses in vivo is not possible and that thereby the absence of liquefaction in infarcts is satisfactorily explained. These observations and conclusions agree well with the results of recent investigations of Waldschmidt-Leitz and his co-workers on cancer in rats, a work to which Hueper contributed the histopathologic part. The necroses were practically free from cathepsin.

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## Notes and News

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**Ella Sachs Plotz Foundation.**—During its tenth year this foundation made twenty-one grants, twelve of which were to scientists outside of the United States. During the present great need for aid, "grants will be given in the sciences closely related to medicine without reference to special fields." The maximum granted will as a rule be less than \$500. Applications for grants for 1934-1935 must be in the hands of the secretary, Dr. J. C. Aub, 695 Huntington Avenue, Boston, Mass., before May 1, 1934.

**Congratulatory Volume.**—Supplement XVI of *Acta pathologica et microbiologica Scandinavica* is dedicated to John Forssman, the discoverer of heterophile antibodies, on his retirement at the age limit from the professorship of general pathology and bacteriology in the University of Lund, Sweden. The supplement covers 611 pages and contains 51 articles by pupils and friends of the dedicatee.

**Society News.**—At the last annual session of the Society of American Bacteriologists in Philadelphia, Milton J. Rosenau was elected president; Karl F. Meyer, vice-president and James M. Sherman, secretary.

The next annual meeting of the American Association of Physicians will be held at the Chalfonte—Haddon Hall, Atlantic City, May 1 and 2, 1934.

The fiftieth session of the American Association of Anatomists is to be held at the University of Pennsylvania, Philadelphia, March 29 to 31, 1934.

The second annual conference of the Society for the Prevention of Asphyxial Death was held in New York on February 19. In addition to papers on the scientific and practical aspects of asphyxia there was a display of appropriate scientific and technical exhibits.

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### CORRECTION

In the abstract of an article by Dr. A. Feller and Dr. H. Sternberg (*Virchows Arch. f. path. Anat.* **285**:112, 1932), which appeared at the top of page 111 in the January issue, the name of a syndrome was misspelled. This should be "Klippel-Feil" instead of "Kippel-Weil."

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

THE INCISOR TEETH OF ALBINO RATS AND GUINEA PIGS IN VITAMIN A DEFICIENCY AND REPAIR. S. B. WOLBACK and P. R. HOWE, *Am. J. Path.* **9**:275, 1933.

The initial effect of a deficiency in vitamin A on the incisor teeth of rats and guinea-pigs is on the enamel organ. The ameloblasts respond earliest by atrophy, and then the remainder of the organ atrophies; finally, metaplasia, calcification and, in the guinea-pig, ossification occur. Atrophy and depolarization of odontoblasts follow changes in the enamel organ. The odontoblasts survive longest on the side (labial) in apposition to the enamel organ. In long continued experiments, gross deformities in the incisors of rats resulted from absence or deficiency of dentine formation. Two types of denticle formation are described, one built up by depolarized odontoblasts, the other by inclusions of ameloblasts made by the folding of imperfectly formed dentine at the formative end of the tooth. Defective formation of enamel and other poorly understood conditions in teeth, such as denticles, pulp bone and cementicles, may reasonably be regarded in the human being as possibly due to deficiency of vitamin A. Our observations indicate that in the incisor teeth of rodents the odontoblasts are influenced throughout life by the enamel organ. As in other morphologic problems concerning deficiencies in vitamins, study of the sequences of repair was essential. We emphasize the importance of two types of material for control, the normal and the progressive stages in repair. Our observations indicate that deficiency in vitamin A is the most important of the known vitamin deficiencies in its effect on the formation of teeth.

FROM AUTHORS' SUMMARY.

EXPERIMENTAL PRODUCTION OF RHEUMATIC LESIONS IN THE HEART AND IN THE JOINTS. ERNST VAUBEL, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:374, 1932.

By repeated subcutaneous or intracutaneous injections of horse serum, morphologic changes were produced in the heart and in the joints of rabbits. In the heart, degeneration of muscular tissue, nodular perivascular cell proliferation and proliferation of the intimal layer of blood vessels were observed. The liver showed degeneration of the parenchyma and proliferation of the reticulo-endothelial tissue. Intra-articular injection of foreign protein caused allergic inflammation of the joints. Periarticular injection produced marked inflammation of all parts of the knee joint. In sensitized rabbits, repeated cooling of the joints may produce severe anaphylactic arthritis with necrosis of the skin. Mechanical injuries of the knee joint may produce in sensitized animals allergic periarticular inflammation. The microscopic lesions of the joints resembled the typical changes of rheumatism in man.

C. ALEXANDER HELLWIG.

EXPERIMENTAL STUDIES ON THE PATHOGENESIS OF JAUNDICE CAUSED BY PHENYLHYDRAZINE, TADASU ITOH, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:513, 1932.

Adult dogs were given injections of a 5 per cent alcoholic solution of phenylhydrazine. At certain intervals the content of bilirubin in the blood serum and the number of red blood cells were determined. The degree of hemolysis did not correspond to the icteric index. Hemolysis cannot be regarded, therefore, as a cause of the jaundice produced by phenylhydrazine poisoning. After repeated injections of phenylhydrazine, central necrosis of the hepatic cells was observed. These

experiments suggest that phenylhydrazine acts in the same manner as removal of the liver, and that the jaundice caused by this poison is due to retention of bile pigment and not to hemolysis.

C. ALEXANDER HELLWIG.

EFFECT OF ARSENIC ON LYMPHOID TISSUE WITHOUT GERMINAL CENTERS. OTTO ISELI, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:529, 1932.

In continuation of experiments by von Albertini, Iseli injected arsenic into very young guinea-pigs, in which the lymph nodes are without so-called germinal centers. A single injection of from 1.3 to 1.8 mg. of arsenic produced a diffuse cell degeneration throughout the lymph nodes, whereas lymph nodes with germinal centers show degenerative changes only in the latter. Repeated injections of small doses of arsenic caused only a moderate decrease of lymphoid cells in the lymph nodes without germinal centers. Iseli believes that his experiments corroborate the theory of von Albertini that the so-called germinal centers are protective organs against poisons carried in the blood stream to the lymphoid tissue.

C. ALEXANDER HELLWIG.

EXPERIMENTAL PNEUMONIA. C. KRAUSPE and J. THIESS, *Beitr. z. path. Anat. u. z. allg. Path.* **91**:276, 1933.

The lobar pneumonias that the authors were able to produce experimentally in normal, immune and sensitized rabbits lead them to conclude that human lobar pneumonia is not a hyperergic inflammation. They believe, however, that variations in the degree of immunity or sensitization at the time of infection by the pneumococcus may be an important factor in determining the variable histologic character of lobar pneumonia in man.

O. T. SCHULTZ.

INFLUENCE OF DIET AND HORMONES ON THYROID FUNCTION. H. PAAL and H. O. KLEINE, *Beitr. z. path. Anat. u. z. allg. Path.* **91**:322, 1933.

The thyroid function in rats was studied. The object was the preparation of a standard diet that would have no effect on the thyroid gland and on which rats might be maintained for the purpose of studying the effects of other factors on the gland. The salt and vitamin content was kept adequate, and wide variations were made in the proportions of protein, fat and carbohydrate. The thyroids of twenty animals in each series were united and extracted, and the protective action of the thyroid substance against acetonitrile was determined on mice. The thyroids of four animals of each series were examined microscopically. In their histologic studies the authors differentiate between the resting gland with storage of colloid and the active gland with secretion of colloid into the follicle and excretion out of the follicle, either of which processes may predominate over the other. Unbalanced diets activated the thyroid, a high content of protein or fat influencing excretion more than secretion, whereas a diet high in carbohydrate caused secretion to predominate over excretion. Submaximal variations in diet led to the storage of thyroid substance without histologic evidence of activity. Iodine compounds brought glands rendered active by diet into a resting state, the degree of storage depending on the dosage. Thyroxine, the hormone of the urine of pregnant women and extracts of the adenohypophysis activated the thyroid, but when thyroxine and pituitary extract were given together, the former inhibited the activating effect of the latter. Vitamin E deficiency inhibited thyroid activity and diminished the activating effect of extracts of the adenohypophysis. Iodine overcame the inhibiting action of vitamin E deficiency.

O. T. SCHULTZ.

EXPERIMENTAL NONINFECTIOUS ARTHRITIS. DSCHU-YÜ-BI, *Beitr. z. path. Anat. u. z. allg. Path.* **91**:361, 1933.

In experiments to determine the character of hyperergic inflammation of joints, rabbits received repeated small doses of horse serum. Only when the injections

were made directly into the cavity of the joint did such inflammation of the cartilage and capsule occur. The inflammation was productive in character and resembled that of rheumatic arthritis, but lacked Aschoff bodies and other histologic characteristics of the rheumatic process. The injection into the joint of non-sensitizing liquids, such as water and physiologic solution of sodium chloride, also caused inflammation of the joint.

O. T. SCHULTZ.

EFFICIENCY OF THE KIDNEY IN COMPENSATORY HYPERTROPHY. H. STAHR, *Centralbl. f. allg. Path. u. path. Anat.* **57**:1, 1933.

The author believes with others that the straight tubules of the kidney are more capable of regenerating than are the convoluted tubules. He presents evidence to prove that a single kidney with compensatory hypertrophy cannot withstand emergencies as well as two normal-sized kidneys. He cites the incompetence of the single enlarged kidney in the following conditions: replacement of one kidney by hypernephroma; agenesis of one kidney; hydronephrosis; stone formation in and destruction of a kidney; disappearance of one kidney due to vascular disease; hypoplasia of one kidney; congenital cystic kidney, and tuberculosis of one kidney.

GEORGE RUKSTINAT.

INJURY TO THE BLOOD VESSELS BY CROTALIN. K. APITZ, *Centralbl. f. allg. Path. u. path. Anat.* **57**:273, 1933.

Ecchymoses were noted in the mesentery of a mouse from five to ten minutes after it had been painted with a 1:1,000 solution of crotalin. The size of the extravasations and the speed with which they developed were noted through a window in the mesentery. After the mesentery was painted with the poison, the circulation continued, and in from three to five minutes red blood cells escaped from the capillaries. The blood gushed out from arterioles and capillary arterioles with "volcanic" force, so that often the serosa tore. When the serosa remained intact, blood soon spread beneath it to obscure the entire field. Where the circulation was only moderately active, free blood cells occurred in grapelike clusters, and subsequent hemorrhage slowly added more red cells to these. From venous capillaries, single red blood cells were extruded at intervals of about a minute. The bleeding did not seem due to inflammation, because stasis and reversal of capillary flow occurred only after bleeding had taken place.

In frogs, crystals of the poison laid on the mesentery slowed the blood stream in the underlying vessels, but bleeding occurred only after the crystals had dissolved. The bleeding was not increased when the pressure in the mesentery was increased by pressing on it with a glass needle.

Sections of mesentery of rats, mice and guinea-pigs failed to disclose alterations in the endothelium of the blood vessels after varying doses of crotalin had been injected intraperitoneally. The conclusion is reached that crotalin is not primarily cytolytic for endothelium but derives hemoglobin, an activating substance, from the wall of the vessel, and in the proper serum medium exerts its injurious effect.

GEORGE RUKSTINAT.

THE BLOOD FORMATION AND THE COPPER CONTENT OF THE CHICKEN EMBRYO. S. SÜMEGI, *Frankfurt. Ztschr. f. Path.* **43**:565, 1932.

The copper content of chicken embryos during incubation increases gradually up to the time of the onset of respiration. Beginning from the onset of respiration, the copper content increases more rapidly. The number of red blood cells and their hemoglobin content are in direct proportion to the copper content of the embryo. The morphologic maturity of the blood cells is also in direct proportion to the copper content. Twenty-four hours after the chicken is hatched, the copper reserve is markedly diminished.

O. SAPHIR.

EXPERIMENTAL PRODUCTION OF LEUKEMIA AND LYMPHOSARCOMA IN MICE BY CHRONIC POISONING WITH INDOL. W. BÜNGELER, Frankfurt. *Ztschr. f. Path.* **44**:202, 1933.

Various doses of a watery solution of indol were injected subcutaneously into a large number of mice. A single injection caused an inhibition of respiration in tissue examined supravitaly. Injections over a long period produced, in addition, increased fermentation. Artificially produced foci of regeneration healed readily and did not, as a rule, precede tumor formations. In only three instances, a small papilloma was found. Severe anemia and leukopenia developed in a large number of animals. Autopsy disclosed gelatinous atrophy of the bone marrow and atrophy of the blood-forming organs. Animals which survived these changes revealed later reparative hyperplasia of the blood-forming organs and distinct leukocytosis in the circulating blood. Of 594 mice, only 97 survived after a period of eight months. Aleukemic lymphadenosis developed in 4 of these, in 1 presenting the histologic appearance of a lymphosarcoma. Myelosis developed in 13 of the 97 animals (4 revealed leukemic myelosis and 9 aleukemic myelosis). The remainder of the 97 animals showed, at autopsy, marked atrophy of the bone marrow, spleen and lymph nodes, and sometimes also severe amyloid infiltration in these organs, in addition to much fibrosis. The author believes that the reason why some animals showed anemia and atrophy of the blood-forming organs, and others hypertrophy and tumor formation, lies in constitutional differences. The majority of the 80 remaining animals, however, showed extramedullary blood-forming foci in the liver, spleen and lymph nodes. In blood smears of these animals, leukocytosis was often demonstrated. Because of the tumor-like proliferations in extramedullary blood-forming organs and because of the low respiration and marked formation of lactic acid in the surviving tissues, it is much more likely that leukemia is a tumor rather than a simple systemic hyperplasia of the blood-forming organs.

O. SAPHIR.

EFFECT OF THE HORMONE OF THE SUPRARENAL CORTX ON THE GENITAL SYSTEM. S. KAPLAN, Frankfurt. *Ztschr. f. Path.* **44**:302, 1932.

From a series of experiments, the author concludes that the hormone of the suprarenal cortex has an inhibitory effect on the genital system of both the male and the female. The lipoid component of the hormone, however, had, to a slight degree, a stimulating effect on the sex organs of the male.

O. SAPHIR.

INTRATHYROID PARATHYROID TUMOR AND GENERALIZED FIBROCYSTIC OSTEITIS. H. SCHLESINGER and E. GOLD, Klin. Wchnschr. **12**:784, 1933.

The conditions indicated by the title were observed in a woman aged 42. The onset of the disorder was associated with pregnancy. After surgical excision of the parathyroid tumor, the excretion of calcium in the urine diminished transiently to zero.

EDWIN F. HIRSCH.

FUNCTIONATING IPSIHOMOGENEOUS TESTICLE (CAT) TRANSPLANT SURVIVING EIGHT YEARS. B. ROMELIS, Klin. Wchnschr. **12**:1640, 1933.

The surviving tissues were mainly Leydig cells.

EDWIN F. HIRSCH.

BONE CHANGES IN EXPERIMENTAL ACIDOSIS. L. HASLHOFER and R. P. CUSTER, *Virchows Arch. f. path. Anat.* **289**:332, 1933.

Katase and his co-workers, by the administration to growing young rabbits of an excess of cane sugar in addition to the regular diet, caused osseous changes that they considered similar to those of osteitis fibrosa and that they believed to be the result of acidosis caused by the high intake of carbohydrates. In the experiments here reported, growing rabbits received 3.5 Gm. of dextrose sub-

cutaneously twice daily for periods of from thirty-three to eighty-five days. The primary and essential changes that occurred in the bones were similar to those of human and experimental rickets. The fibrotic changes described by the Japanese observers, the present authors hold to be secondary and the result of mechanical factors acting on the weakened bones.

O. T. SCHULTZ.

EXPERIMENTAL CORONARY SCLEROSIS. W. MOSEBACH, *Virchows Arch. f. path. Anat.* **289**:647, 1933.

Pfleiderer, working in Schmidtman's laboratory, showed that it is possible to produce typical atherosclerosis of the coronary arteries in rats by combining the feeding of cholesterol and vitamin D (viosterol) with daily exercising of the animals in a treadmill. The purpose of the present work, which was also done in Schmidtman's laboratory, was to evaluate the rôle of the three factors, cholesterol, vitamin D and functional activity, in the development of the coronary sclerosis. It was found that neither work alone nor work combined with the feeding of cholesterol caused disease of the coronary arteries. Vitamin D is necessary; it can be replaced by epinephrine but not by irradiation of the animals.

O. T. SCHULTZ.

TISSUE CULTURE OF SYNOVIAL MEMBRANE. E. VAUBEL, *Virchows Arch. f. path. Anat.* **289**:670, 1933.

After a brief review of the varying opinions that have been and are still held regarding the histologic structure and nature of the synovial membrane and the origin of synovial fluid, the author presents the results obtained by tissue culture. Cultures of serous membranes and other mesenchymal tissues from the same animal, the rabbit, were also studied. Synovial membrane is mesenchymal in origin, but in culture it differs in type of growth and in cell function from other mesenchymal tissue. The actively growing cells are elongated and have a granular cytoplasm, the granules staining deeply with toluidine blue and neutral red. The cells form a syncytial network in which appear spaces formed by plasmolysis brought about by a proteolytic enzyme. About the spaces the cells become polyhedral or cuboidal and may form a closed, regular layer similar to epithelium. The spaces are filled with a mucoïd fluid, and the network of tissue with its fluid-filled spaces has the appearance of embryonic mucoïd tissue. The fluid is looked on as the ground substance of the synovial tissue. Coalescence of the spaces leads to the formation of the joint cavity. Because of the specialized functions of the growing cells derived from synovial membrane, the author proposes for them the name "synovioblast."

O. T. SCHULTZ.

PHYSIOLOGY OF THE HYPOPHYSIS. J. B. COLLIP, H. SELYE and D. L. THOMPSON, *Virchows Arch. f. path. Anat.* **290**:23, 1933.

In this article Collip and his associates at McGill University present a succinct and well organized summary of experimental work on the hypophysis that has appeared in briefer form in American and Canadian journals. They describe a method of approach for the removal of the hypophysis through the base of the skull that does not necessitate opening of the pharynx and that they consider superior to other operative procedures. The rat has been the experimental animal. With hypophysectomized rats as the test object, it has been possible to study the effects of various extracts of the pituitary. It has been possible to separate at least three active principles, a growth hormone, a sex hormone and a thyrotropic hormone. Still another principle, which prevents or overcomes the atrophy of the suprarenal cortex which follows removal of the hypophysis, they believe to be distinct from the other three hormones. They present evidence that prolan, the sex hormone isolated from the urine of pregnant women, is not identical with the pituitary sex hormone. In sexually immature rats, prolan causes theca luteinization

of the ovarian follicles but not maturation of follicles or the formation of true corpora lutea, whereas the sex hormone prepared from the pituitary does have the two last named actions. In the male animal, prolan causes hyperplasia of the interstitial tissue of the testis and increase in the size of the accessory sex glands, but does not lead to regeneration of the tubular epithelium. The pituitary sex hormone effects regeneration of seminal epithelium and renewed spermatogenesis. Hypophysectomy during the second half of pregnancy indicates that the pituitary is not necessary for the continuation of pregnancy, the normal birth of the young and the initiation of milk secretion. In such animals lactation, although established, ceases within a few days of the birth of the young. In the normal, nonpregnant female, administration of prolan followed by castration leads to the secretion of milk. If, in such animals, the hypophysis is also removed at the time of castration, lactation does not occur.

O. T. SCHULTZ.

INFLUENCE OF CYTOTOXINS ON THE GROWTH AND METABOLISM OF TISSUE CULTURES. N. N. SPASSKY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 77:365, 1932.

Two types of cytotoxic serums were employed, one of which was produced by immunizing rabbits with splenic tissue of guinea-pigs; the other was directed against the tissues of chick embryos. Some of the serums arrested or inhibited the growth of the homologous tissues. The cellular metabolism, as measured by the decrease of the sugar content of the culture, was only moderately lowered. This indicates that the damage to the cells of the tissue culture was not very far-reaching and, furthermore, that the amount of energy necessary for the maintenance of vital activities is much greater than the amount consumed for growth.

I. DAVIDSOHN.

QUININE AND ULTRAVIOLET RAYS. O. JIROVEC and V. BOUŠE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 78:100, 1933.

The toxicity of solutions of quinine, acriflavine, eosin and pyronine for paramacia did not increase following irradiation of the solutions with ultraviolet rays. This contradicts the reports of Roskin and Romanowa, who had observed a marked increase of the toxic effect of quinine on paramacia following irradiation.

I. DAVIDSOHN.

COMBINED ACTION OF ARSPHENAMINE AND ULTRAVIOLET RAYS ON SPIROCHAETA PALLIDA. S. S. ORLOW and L. B. LEWINSON, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 78:264, 1933.

Rabbits were given combined treatment with arsphenamine and ultraviolet rays after the development of primary syphilitic lesions. The fur on the abdomen was removed and an erythema dose given (usually from three to five minutes at 25 cm.) immediately after the injection of neoarsphenamine and again on the following day. The result was a marked increase in the therapeutic effect and a shortening of the period of illness. The spirochetes in the primary lesion disappeared earlier than in the controls. The toxic effect of the drug was not increased. Orlow and Lewinson advocate a wider application of the combined treatment in the clinic.

I. DAVIDSOHN.

FAMILIAL OCCURRENCE OF LEUKEMIA. SVEND PETRI, *Acta path. et microbiol. Scandinav.* 10:330, 1933.

The literature is reviewed, and thirty-three cases of familial leukemia are described, of which eleven are beyond doubt. The lymphatic type occurred in twenty-two instances. There was an average of three cases in a family. The author adds a



report on chronic lymphatic leukemia in two brothers. There is no evidence of hereditary transmission of leukemia, although there is some endogenic factor associated. The presence of infection or of a common external agent could not be ruled out. The possibility of mere coincidence in "familial" leukemia must be considered. Lymphatic leukemia occurred in 85 per cent of related persons, while myelogenous leukemia occurred in nonrelated persons.

JACOB KLINE.

### Pathologic Anatomy

CORONARY EMBOLISM. O. SAPHIR, *Am. Heart J.* 8:312, 1933.

The literature on coronary embolism is reviewed and the rarity of such an occurrence emphasized. Cases in which the source of the embolism is not found at autopsy should not be accepted as proved cases of coronary embolism. Three cases of coronary embolism are reported. In one, the source of the embolism was a thrombus in the femoral vein; there was also a patent foramen ovale. In the second case, the source of the embolus was a mural thrombus in the right coronary artery, occurring on the basis of an atheromatous ulcer. The embolus had lodged in the distal part of this artery at the origin of the posterior descending branch. In the third instance, the source of the embolus which had occluded the mouth of the right coronary artery was a thrombus occurring on an atheromatous ulcer in the region of the sinus of Valsalva. In all three instances the patients died suddenly.

AUTHOR'S SUMMARY.

MULTIPLE RUPTURE OF THE HEART BY INDIRECT TRAUMA. O. SWINEFORD, *Am. Heart J.* 8:418, 1933.

A unique instance of multiple rupture of the heart caused when an automobile struck the patient from behind is presented. One rupture was of the interventricular septum, one was of the anterior wall of the right ventricle and one was of the interauricular septum.

RELATIONSHIP OF THE RHEUMATIC PROCESS TO THE DEVELOPMENT OF ALTERATIONS IN TISSUES. ALVIN F. COBURN, *Am. J. Dis. Child.* 45:933, 1933.

The observations at necropsy are presented in six cases of rheumatic fever in the active stage. The diagnosis was established in each instance by the characteristic early specific lesion in the endocardium of the mitral valve and left atrium. The conspicuous lesions, however, consisting of numerous focal hemorrhages in the viscera and beneath the serosal surfaces, might be considered as nonspecific, but are here looked on as specific also. In the lungs were focal areas of vascular engorgement, subpleural and alveolar hemorrhage and massive accumulations of large mononuclear cells. Polymorphonuclear leukocytes were rarely seen, and micro-organisms were not detectable in these lesions. There were no apparent changes in the blood vessels. These anatomic findings indicate that in addition to the well recognized swelling of endothelium and fragmentation of collagen, diffuse hemorrhagic changes may be characteristic of the active rheumatic process.

RALPH FULLER.

CYSTS AND DIVERTICULA OF INTESTINAL ORIGIN. HENRY G. PONCHER and GEORGES MILLES, *Am. J. Dis. Child.* 45:1064, 1933.

A case of enterogenous cysts of the mediastinum containing gastric mucosa and an intramesenteric cyst of the ileum containing gastric mucosa is reported. Attention is redirected to the epithelial nodes and diverticula reported by Lewis and Thyng as a probable source of origin for these rare anomalies.

FROM AUTHORS' SUMMARY.

NIEMANN-PICK'S DISEASE AND OTHER FORMS OF SO-CALLED XANTHOMATOSIS.  
LUDWIG PICK, Am. J. M. Sc. **185**:601, 1933.

The knowledge of the generalized essential xanthomatoses is still recent in its present classification. The important information regarding the nature of Gaucher's, Niemann-Pick's and Hand-Schüller-Christian's diseases dates back only to the last decade. There is, however, no doubt in regard to the principal differences among these three conditions. The separation of these three storage diseases from one another is the more certain because the differentiation is not only clinical and anatomic but also chemical. Gaucher's disease stores mainly kersasin; Niemann-Pick's disease, phosphatides, and Hand-Schüller-Christian's disease, cholesterol. It is true that certain features are common to all three diseases. In particular, there is a familial congenital disturbance of the lipoid metabolism on a constitutional basis, and this aberration shows predilection for the Jewish race. The reason for this is probably the coincidence of the determining hereditary factors favored by frequent blood marriages. All three diseases are accompanied by hemachromatosis with yellowish-brown pigmentation of the exposed skin. In all three diseases, disappearance of the storage cells may occur, with the subsequent formation of fibrous scars. Gaucher's disease and Hand-Schüller-Christian's disease exhibit surprisingly independent localization of lipoid storage within the skeletal system. This occurs apparently in still another form, which is at present still classified among the unnamed essential xanthomatoses. Yet there is, aside from this fundamental agreement, almost always a particular characteristic for each disease entity. Gaucher's disease and Niemann-Pick's disease predominate in the female sex; Hand-Schüller-Christian's disease predominates in the male sex, as far as the existing observations show. In Gaucher's disease and in Niemann-Pick's disease the cicatricial new formations develop in the liver, with disappearance of the storage cells directly by a sort of insidious condensation of the fibrous stroma. In Hand-Schüller-Christian's disease, however, and also in the unnamed xanthomatoses of the bone there is formation of granulation tissue. In Gaucher's disease the osseous form of the disease is exceptional. In Hand-Schüller-Christian's disease and also in the unnamed xanthomatoses of the bone, it is the rule and the leading symptom. In Hand-Schüller-Christian's disease the skull is involved primarily; in Gaucher's disease and in the xanthomatoses of the bone, the entire skeleton.

AUTHOR'S SUMMARY.

TUBERCULOUS VEGETATIONS OF THE TRUNK OF THE PULMONARY ARTERY.  
P. GROSS, Am. J. Path. **9**:17, 1933.

A third case of tuberculous involvement of the main stem of the pulmonary artery has been described. The tuberculous involvement in this case is the result of the extension of tuberculous lymphadenitis from adjacent lymph nodes to the adventitia and thence to the intima of the pulmonary artery.

AUTHORS' SUMMARY.

MICROINCINERATION STUDIES OF HUMAN CORONARY ARTERIES. D. Y. KU,  
Am. J. Path. **9**:23, 1933.

The method of incineration was employed in the study of the smaller divisions of the coronary arteries in sixty-two patients ranging in age from new-born infants to persons aged 79. Ash was demonstrated in all, but in variable amounts. The smallest amount was found in the arteries of new-born infants. This amount gradually increased during the first, second and third decades, apparently as a corollary of normal growth. The increase in the amount of ash after the third decade was definite, but was without regularity of progress. This total variation, associated with individual variation, was probably the result of arteriosclerosis in these higher age groups. It was found also that rheumatic fever nodules and leukemic infiltrates increased the amount of ash. The ash of coronary arteries is derived principally from the elastic fibers, but also in small part from the intima, media

and adventitia. This should be regarded as a normal finding, but not of necessity as a precursor of arteriosclerosis. The ash is composed almost entirely of calcium salts, but the manner in which these salts are bound to the elastic fibers is not known. As the quantity of elastic tissue increases normally and as it increases as a part of arteriosclerosis, there is an increase in the amount of ash. It is suggested but not proved that alteration in quantity and quality of elastica may have some bearing on the calcification that occurs in the course of arteriosclerosis.

AUTHOR'S SUMMARY.

METASTATIC CALCIFICATION IN MYELOGENOUS LEUKEMIA. D. A. DESANTO, *Am. J. Path.* 9:105, 1933.

A case is reported of metastatic calcification occurring in a young girl whose history indicated that she had had myelogenous leukemia for at least two years. The term is employed to designate a condition in which calcium is mobilized from the depots in the bones and reprecipitated in other tissues of the body, particularly elastic tissues, which are probably physically constituted to favor calcium adsorption and reprecipitation. Calcium deposition is also seen to be favored by any phenomenon which reduces the acidity of the tissue fluid. Hence, metastatic calcification occurs in the tissues in which a change in the alkaline direction likely occurs. Recent evidences mentioned show that the leukemias are rarely associated with osteolytic changes in the skeleton, and this infrequency probably explains the rarity of metastatic calcification as a finding in leukemias. Finally, the identity of this condition with the calcifications occurring after the administration of parathormone and viosterol is reaffirmed.

AUTHOR'S SUMMARY.

AMYLOID DISEASE OF THE KIDNEYS. E. T. BELL, *Am. J. Path.* 9:185, 1933.

A study of sixty-five cases of amyloid disease of the kidneys is reported. These are arranged in four groups corresponding roughly with the degree of glomerular involvement. In groups A and B the symptoms are essentially those of the underlying infection, and with few exceptions albuminuria or edema is the only symptom referable to the kidneys. In group C there is some impairment of renal function, and in group D there is evidence of advanced renal insufficiency. Albuminuria is rarely absent, but the amount of albumin does not indicate accurately the extent of the amyloid deposit. Edema is a variable feature with no evident relation to the degree of renal damage. Hypertension is occasionally found in amyloid disease with renal insufficiency. It is probably due to obstruction in the arteriolar and glomerular circulation. Renal insufficiency is a frequent cause of death. It is caused chiefly by amyloid deposits in the glomerular capillaries, but obstruction of the tubules by casts and amyloid deposits in the medulla, around the tubules and in the arterioles, is often an important factor in the production of uremia. In the glomeruli amyloid is deposited on the inner surface of the capillary basement membrane. Endothelial nuclei are frequently displaced inwardly and become scattered through the amyloid. The capillaries usually become greatly distended with amyloid, and they may remain permeable in the presence of massive deposits. The glomerular epithelium degenerates and is desquamated. There is commonly a definite increase of endothelial nuclei in the glomerular capillaries preceding the deposit of amyloid. This is attributed to the underlying infection. It is not sufficiently prominent to be identified with clinical acute glomerulonephritis. Amyloidosis is a special form of renal disease. There is no advantage in classifying it as nephrosis. A sharp distinction between nephrosis and nephritis has not been established. Amyloidosis is a primary renal disease. It is rarely a complication of a preexistent clinical renal lesion.

AUTHOR'S SUMMARY.

SPONTANEOUS RUPTURE OF THE HEART. R. L. BENSON, W. C. HUNTER and C. H. MANLOVE, *Am. J. Path.* 9:295, 1933.

Forty cases of rupture of the heart have been collected from nearly 7,000 autopsies in Portland, Ore. One rupture was probably of syphilitic origin. Another, a

dissecting aneurysm of a sinus of Valsalva, was due to endocarditis caused by *Streptococcus viridans*. The remaining 38 ruptures, although in some instances manifesting evidence of syphilis, were attributable to recent or old thrombosis, embolism or arteriosclerosis of the coronary arteries. AUTHORS' SUMMARY.

THE CELLULAR REACTION IN TUBERCULOSIS OF THE CORNEA. E. R. LONG, S. W. HOLLEY and A. J. VORWALD, *Am. J. Path.* 9:329, 1933.

Central interlamellar corneal injections of from 0.01 to 0.005 mg. of moderately virulent tubercle bacilli of human type were made in a series of normal and tuberculous guinea-pigs, and normal rabbits and cats. Animals of each kind were killed at six hours, one, three and fourteen days and one month, and the nature and extent of the inflammatory response were determined by microscopic examination. In all cases the first reaction noted was at the limbus, and not at the site of injection. The reaction consisted of an outpouring of polymorphonuclear leukocytes from the marginal vessels. It was most intense in the tuberculous guinea-pig (tuberculin reaction), less in the normal guinea-pig, still less in the rabbit and least in the cat. The first cellular reaction at the site of injection in the center of the cornea consisted of leukocytes migrating in from the margin. The reaction was well developed in all animals at twenty-four hours, and the cells taking part were almost exclusively polymorphonuclear leukocytes. After twenty-four hours there was a gradual increase in the proportion of large mononuclears in the reaction. The rate of increase rose with the closer approach of blood vessels, as vascularization of the cornea developed. The paucity of these cells at the earlier stages and the abundance in the vascularized state of the cornea seemed good evidence that the large mononuclear leukocyte in corneal tuberculosis is not locally derived, as formerly claimed. In all animals the large mononuclears, as they reached the site of infection, engulfed the polymorphonuclear leukocytes already there, taking over the tubercle bacilli contained by the latter cells. During the period from three days to two weeks after the injection the inflammatory reaction progressed most rapidly in the tuberculous guinea-pigs, and less rapidly in the normal guinea-pigs, rabbits and cats in the order named. Ulceration occurred in the tuberculous guinea-pigs in two weeks, and these animals were dropped from the series at this point. At one month, the time of the last examination, the intensity of inflammatory reaction, as determined by the size of the lesion and the proportion of the cornea involved, was greatest in the guinea-pig, less in the rabbit and least in the cat. This order is in inverse relation to the general resistance of these animals to the strain of tubercle bacillus used. AUTHORS' SUMMARY.

THE ORIGIN OF THE EPITHELIOID CELL IN TUBERCULOSIS OF THE CORNEA. E. R. LONG and S. W. HOLLEY, *Am. J. Path.* 9:337, 1933.

Infection of the center of the rabbit cornea with tubercle bacilli of human type resulted in a lesion characterized almost solely by polymorphonuclear leukocytes until the arrival of new capillaries, when a rapid replacement of the polymorphonuclears by mononuclear phagocytic cells occurred. When two infecting doses were placed in the same cornea, one at the margin near the normal blood supply and one in the normally avascular center, the replacement of polymorphonuclears by large mononuclears occurred weeks earlier in the lesion close to the limbus. The mononuclears accounting for this replacement and functioning in the subsequent development of the lesion apparently arose from primitive smaller mononuclear cells present in large numbers in and around the walls of the new capillaries. The source of these cells could not be determined with absolute certainty, but the following observations were evidence that most of them came from the blood stream: Cells of similar character were present among the erythrocytes in the lumens of the blood vessels in great excess of the normal proportion; migration figures of similar cells could be seen in the walls of the growing capillaries, and mitotic figures among them were rare. The primitive cells, after accumulating

in and around the walls of the capillaries, underwent a progressive transformation by simultaneous increase in size and change of character, without mitosis, into epithelioid cells.

AUTHORS' SUMMARY.

FIBROSIS OF THE UTERUS. A. B. BAKER, *Am. J. Path.* 9:369, 1933.

With the advance of age, there occurs within the uterine wall a gradual increase of collagenous fibers that ultimately replace the greater part of the muscle. Neither localized nor acute systemic processes have any effect on the uterine wall. Chronic diseases have no effect on the uterine wall of young nulliparas, but hasten the fibrotic process in older nulliparas. The uterine arteries show less pronounced change with age than the uterine muscles. The three alterations most commonly found are medial fibrosis, medial calcification and intimal atherosclerosis. There is no satisfactory evidence that either fibrosis of the uterine wall or increase in elastic tissue following pregnancy is responsible for uterine hemorrhage.

AUTHOR'S SUMMARY.

SEVEN PREHISTORIC AMERICAN SKULLS WITH COMPLETE ABSENCE OF AN EXTERNAL AUDITORY MEATUS. A. HRDLICKA, *Am. J. Phys. Anthropol.* 17:355, 1933.

Complete congenital absence of the external auditory meatus and of the tympanic bone was observed in seven skulls of pre-Columbian or old American aborigines. These are the first cases of this nature recorded in persons of this race. The anomaly predominates in females, at least five and probably six of the seven subjects having been of that sex; it is unilateral in all the specimens, and in every one of the seven skulls it exists on the right side. Five of the skulls are from the coast of Peru; one is from a mound in Arkansas, and one from a cave in New Mexico. Two of the five Peruvian skulls come from one moderate-sized burial ground, which suggests close relationship. The anomaly, serious as it is, has produced remarkably few effects on the base of the skull; yet some effects are visible. One of these is a perceptibly smaller development in nearly all of the skulls of the petrous portion on the affected side; another is the absence of the styloid process on the side of the defect in most of the skulls; the third is the presence on the abnormal side, in about the midst of the glaserian fissure, of one or two canals, in some cases of fair size, which are absent on the sound side. The left meatus, while of normal conformation in all the cases, in the majority is of submedian capacity, and in one skull, that of a child, its lumen is decidedly subnormal. The anomaly presented by the specimens here reported can be characterized, it seems, only as "agenesis," due either to some germinal defect or to a weakened influence of the trophic nerve centers. It shows such similarity in the relatively large series of cases here reported that it must be regarded not merely as a chance condition, but as a definite syndrome or unit of agenetic nature.

CONGENITAL CYSTIC DISEASE OF THE LUNG. WILLIAM C. POLLOCK and HORACE P. MARVIN, *Am. Rev. Tuberc.* 27:59, 1933.

Congenital cystic disease of the lung is believed to be a definite disease entity of rare occurrence. The cystic condition is secondary to a congenital narrowing of the main bronchus or bronchi of the involved lobe or lobes. The cystic changes are usually more or less stationary, but may be progressive under certain conditions. Usually there is only a congenital defect of the bronchus of one lobe, more frequently the upper lobe. In these cases there may be extensive cystic changes of the entire lobe without symptoms. When more than one lobe is involved, conditions of pulmonary stress by hyperphysical activity may cause a progression of

the cystic disease until such symptoms as dyspnea and cough develop. Cystic disease is usually discovered accidentally by routine examinations. It should not be confused with congenital bronchiectasis, as there are no dilatations of the bronchi of the cystic lung.

H. J. CORPER.

NOCARDIOSIS CUTIS GANGRENOSA. W. H. GUY and T. R. HELMBOLD, Arch. Dermat. & Syph. 27:224, 1933.

Nocardia was found in blood and tissue culture from a patient with a fulminating gangrenous lesion of the breast. Histologic section showed parenchymatous and interstitial edema in the dermis, with a few polymorphonuclear leukocytes. The deeper vessels were thrombosed. There was a wide zone of perivascular infiltration comprised predominately of polymorphonuclear leukocytes with a scattering of lymphocytes, monocytes and plasma cells. A moderate diffuse infiltration of the same character was present in the papillary zone. Minute foci of coagulation necrosis of collagen occurred throughout the section. The elastic tissue was swollen but not disarranged. Gram-Weigert preparations revealed foci of filaments of Nocardia near the vessels, and, in one instance, in a thrombosed vessel. The patient had lost 60 pounds (27.2 Kg.) prior to the onset of the dermatosis. Bloody diarrhea was present, with ulceration of the rectum and sigmoid. The condition was interpreted as an embolic process with dissemination by way of the blood stream, perhaps in this case from the gastro-intestinal tract.

S. W. BECKER.

SUBCUTANEOUS FAT NECROSIS OF THE NEW-BORN. HOWARD FOX, Arch. Dermat. & Syph. 27:237, 1933.

Five cases are reported of localized induration of the subcutaneous tissue in young infants, presumably following trauma subsequent to forceps delivery or to violent measures for resuscitation. The lesions persist for from five weeks to five months and disappear. The disease involves the entire subcutaneous tissue, sparing the epidermis and dermis. The process is essentially a necrosis of the fat cells and an infiltration of epithelioid and giant cells. Needle-like crystals in the form of rosettes or sheaths, possibly composed of fatty acids, are found in the fat cells and in the giant cells. The picture is that of a lipophagic granuloma which undergoes, first, cicatricial organization, and, later, complete restitution to the normal condition with the formation of new fat. The condition must be distinguished from sclerema adiposum, sclerema edematosum and scleroderma. Sclerema and scleroderma are much more serious diseases, although many cases in the literature prior to the last decade reported under these names were probably cases of subcutaneous fat necrosis.

S. W. BECKER.

LOCALIZATION AND DEVELOPMENT OF THE MELANIC CELLS OF THE HUMAN CEREBELLUM. J. ARANOVICH, *Semana méd.* 2:927 (Sept. 28) 1933.

Aranovich states that in the lateral region of the fourth ventricle in the cerebellum of the human being there exists a nucleus of melanic cells, the so-called subependymal-laterocerebellar nucleus. The nucleus is pigmented during the first months of life and presents morphologic differences from the cells of the locus caeruleus and from those of the locus niger. Under normal conditions, the cells of the nucleus are large and rich in pigment and have a well defined nucleus and nucleolus. Similar to other melanic cells, those of the laterocerebellar nucleus undergo characteristic changes in the course of Parkinson's disease (reduction and deformation of the cells, decrease of the pigment, deformation of the nucleus and disappearance of the nucleolus). The clinical and anatomicopathologic study of a case of Parkinson's disease is reported.

## Pathologic Chemistry and Physics

A REDUCING SUBSTANCE IN A CHROMOPHILIC ADENOMA AND IN THE NORMAL ANTERIOR PITUITARY. T. J. PUTNAM and H. B. WILCOX, *Am. J. Path.* **9**:649, 1933.

A substance reducing methylthionine chloride, U. S. P. (methylene blue) has been demonstrated in the normal anterior lobe of the pituitary gland and its extract, and in larger amounts in a chromophil adenoma. None was found in a chromophobe adenoma.

AUTHORS' SUMMARY.

MELANOGENESIS WITH REFERENCE TO SULPHYDRILS AND PROTAMINES. H. J. ROPSHAW, *Am. J. Physiol.* **103**:535, 1933.

The experiments reported here support the view that melanogenesis is an intracellular process and a physiologic function of the pigment cell. It results from the reaction of cystine on protamine, and so in the skin is limited to the epithelial layer—the only one which contains sulphydril compounds. Formation of melanin is an evidence of a phase of nuclear metabolism—the cleavage by which protamine is freed to diffuse into the cytoplasm, with the cystine-cysteine complex serving as an indicator of this reaction. In the cytoplasm the cystine is present within glutathione, and the protamine in the chromatin. Both are freed by enzyme cleavage and react to form melanin when brought in contact. A similar reaction takes place in vitro, progressing rather slowly because of the gradual oxidation of cysteine. It does not occur in an oxygen-free medium, and apparently requires the presence of iron as a catalyst—found in the skin in the protamine. The black precipitate formed in these circumstances becomes white in time, which suggests that melanin may be excreted as colorless succedaneous products. Acidity prevents the reaction, and Ropshaw suggests that there may be a relation between this and albinism.

H. E. EGGERS.

THE QUESTION OF A PRESSOR SUBSTANCE IN THE BLOOD IN ESSENTIAL HYPERTENSION. G. E. WAKERLIN and H. D. BRUNER, *Arch. Int. Med.* **52**:57, 1933.

The action of thirty blood serums from patients with essential hypertension and of fifteen serums from patients with normal blood pressure on the tone of arterial segments from the mesenteric arteries of cattle was studied. No significant differences were found in the vasoconstricting properties of these serums. The results suggest that there is no peripherally acting pressor substance in the blood of patients with essential hypertension. Some evidence was obtained for the residence of a spontaneous rhythmic motor activity in arterial musculature deprived of its extrinsic innervation.

AUTHORS' SUMMARY.

MINERALS IN SILICOTIC LUNGS. W. R. JONES, *J. Hyg.* **33**:307, 1933.

The bulk of the mineral residue found in the lungs examined consisted of minute fibers of sericite, a hydrated silicate of aluminum and potassium. The same mineral was found in the rock dust inhaled. Silica in the uncombined state, quartz, was also found in the residue. However, silica in the uncombined state was not the chief cause of silicosis in these cases. The fibrous minerals present seemed to have hastened the process of silicosis, and their presence in the rock appears to be of more importance than the presence of the quartz.

EDNA DELVES.

A URINARY COMPOUND OF ALBUMIN, BENGE-JONES PROTEIN, PSEUDOGLOBULIN AND AN UNKNOWN ANTIGEN. W. H. WELKER and L. HEKTOEN, *J. Infect. Dis.* **53**:165, 1933.

The urine of a patient with myeloma contained a compound of albumin, pseudoglobulin and Bence-Jones protein and an unknown antigen. The Bence-

Jones protein could not be separated from this compound in pure form by boiling or by attempts at crystallization. Individual precipitins in rabbit serum prepared against the compound and against solutions of substances obtained on attempts at crystallization were removed by specific adsorption. AUTHORS' SUMMARY.

ESTIMATION OF TISSUE PHENOLS. M. I. SMITH, *Pub. Health Rep.* **48**:1487, 1933.

A method has been described for the quantitative estimation of true phenols, free and conjugated, applicable to all body tissues and fluids. The tissues of the normal rabbit (oat and cabbage diet) were found to contain less than 1 mg. per hundred grams of tissue and usually not much over 0.5 mg. of what might be regarded as true phenols. In phenolorthocresol poisoning, phenols were found in appreciable amounts in all the tissues examined. In acute lethal poisoning, free phenol was found in concentrations ranging from about 7 to 26 mg. per hundred grams of tissue, the lowest value being found in skeletal muscle and the highest in the kidney. Conjugated phenols were not found in appreciable amount anywhere. In subacute poisoning, conjugated phenols were found in all the tissues examined, with the exception of the central nervous system, which showed little or no combined phenols. These observations suggest that the function of phenol conjugation is not limited to any one tissue, although it seems to occur predominantly in the kidney, liver and intestine, while the brain and probably also the spinal cord appear to be devoid of this function. As much as from 6 to 8 mg. of free phenol per hundred grams of tissue was found in the central nervous system of the rabbit following the administration of a toxic but nonfatal dose of phenol.

AUTHOR'S SUMMARY AND CONCLUSIONS.

IMMUNOLOGY OF CASEINS. M. L. DEMANEZ, *Arch. internat. de méd. exper.* **8**:233, 1933.

Biologic tests of the caseins from cows, goats and ewes indicate a great similarity in the immune reactions. The caseins of women and mares are quite distinct from the preceding varieties. Casein is a mixture of several proteins, which cannot be distinguished in biologic tests. The specificity of casein is not modified by heat or the action of iodine. In this it differs from protein of blood serum.

JACOB KLEIN.

SPECIFICITY OF FIBRINOGENS. M. L. DEMANEZ, *Arch. internat. de méd. exper.* **8**:255, 1933.

There has been a divergence of opinion as to the immunologic properties of the fibrinogens of the different mammals. By precipitin and absorption tests, the author has tested the fibrinogens of the pig, horse, sheep and cow, and concludes that the fibrinogens of different zoologic species possess only a limited specificity.

JACOB KLEIN.

HISTOCHEMICAL AND MINERALOGICAL ANALYSIS OF DUST PARTICLES IN PNEUMONOCOCONIOSIS. K. F. SCHEID, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:93, 1932.

For identification of dust particles in fifty lungs, these methods were used: examination of frozen sections of fresh tissue, incineration at from 450 to 500 C., tests with chemical reagents (acids, alkalis), refractometric methods and polariscopy. The characteristics of carbon, graphite, magnetite, pyrite, hematite, lime, silicon dioxide and muscovite are described. From the biologic point of view, two forms of dust must be distinguished: (1) minerals which are readily dissolved by the tissue like lime and siderite and (2) minerals which are deposited in the tissue (silicon dioxide, carbon, rutile and glimmer).

C. ALEXANDER HELLWIG.



**SPHERICAL MICROLITHS OF BILE.** G. LEMMEL and W. BÜTTNER, Beitr. z. path. Anat. u. z. allg. Path. **91**:19, 1933.

Microscopic formed particles of variable size, shape, structure and composition were described many years ago in the bile of the gallbladder. Only recently have these microliths begun to attract attention in the current literature. The authors limit their discussion to the spherical microliths, the morphology of which they describe and illustrate by photomicrographs. These microliths have a laminated and often radially striate structure. The smallest ones have a small center and a highly refractile outer portion, and are light green. Larger ones are darker and consist of layers of refractile ground substance separated by thinner layers of bile pigment. The authors interpret the increase in size and the lamination as evidences of growth and age. The presence of older microliths may, therefore, indicate pathologic alterations in the biliary passages that are no longer evident at the time of examination. Older microliths may show the effects of the action of bile on their superficial portions.

O. T. SCHULTZ.

**QUANTITATIVE SPECTROGRAPHIC DETERMINATION OF GOLD IN TISSUES.** WERNER GERLACH, K. RUTHARDT and L. PRÜSENER, Beitr. z. path. Anat. u. z. allg. Path. **91**:617, 1933.

Gerlach's seventh contribution to the elementary chemical analysis of tissues is devoted to gold. There is described a spectrographic method that permits rapid quantitative as well as qualitative estimation of gold. Similar methods for copper, lead and manganese are promised. The advantages and possibilities of the spectrographic method are discussed. For the histochemical detection of gold in tissues, the method of Timm was found to be best, but it failed to reveal small quantities that could be readily determined by the method of spectral analysis.

O. T. SCHULTZ.

**HYPERPROTEINEMIA IN MYELOMA.** M. BÖNNINGER, Deutsche med. Wchnschr. **59**:770, 1933.

A 52 year old man who was ill with generalized myeloma was found to show a marked tendency to coagulation of the blood. This was so extreme that it was impossible to count red cells with Hayem's solution (mercuric chloride, 0.5 Gm.; sodium sulphate, 5 Gm.; sodium chloride, 1 Gm., and distilled water, 200 cc.). The serum was thick and tenacious like acacia, and the blood corpuscles were markedly clumped. There was an increase of the euglobulin in the serum. The urine contained traces of albumin and no Bence-Jones protein.

JACOB KLEIN.

**EFFECT OF X-RAYS ON THE LUNGS AND HEART.** M. I. KARLIN and B. N. MOGILNITZKY, Frankfurt. Ztschr. f. Path. **43**:434, 1932.

The authors believe that too little attention is paid to the possible damage of vital organs in cases of prolonged roentgen treatment of carcinoma of the mammary glands, lungs and other organs. In reviewing the literature, they found a divergence of opinion concerning this possibility. In dogs that were treated with various doses of roentgen rays, peribronchial and perivascular connective tissue proliferations and epicardial thickenings were found. Bronchopneumonia and necrotizing bronchitis were also encountered. Occasionally hemorrhages were seen, especially pronounced in the perivascular spaces and beneath the endocardium. The authors conclude that possible damage of the heart and of the lungs in the human being treated with roentgen rays over a long period of time must be considered.

O. SAPHIR.

INCREASE IN PHOSPHATIDE IN NIEMANN-PICK'S DISEASE. E. EPSTEIN, *Klin. Wchnschr.* **12**:56, 1933.

The saturated and unsaturated phosphatide contents of the spleen, liver and brain cells are greatly increased over the normal in Niemann-Pick's disease. In the brain and liver there is a marked increase in the ratio of free cholesterol to cholesterol ester.

D. O. ROSEASH.

SYNTHESIS OF URIC ACID IN BIRDS. W. SCHULER and W. REINDEL, *Klin. Wchnschr.* **12**:1479, 1933.

The precursors in the synthesis of uric acid in pigeons and geese are found in the liver. Their formation is an enzyme process with an optimum temperature of 41 C. and an optimum  $p_H$  of 7.6. The nitrogen sources of the precursors are amino-acids above ammonia but not beyond urea. Precursors may be extracted from muscle, but a fermentation formation has never been demonstrated. The formation of uric acid in the kidney is optimum at  $p_H$  7.1.

AUTHORS' SUMMARY.

DETECTION OF THORIUM IN TISSUES. W. GERLACH, *Virchows Arch. f. path. Anat.* **287**:135, 1932.

The author gives a brief discussion of the spectrographic detection of thorium in tissues. The thorium lines, at 2,832 and 2,837 angstrom units, are easy to detect because they lie between the prominent magnesium lines that all tissues give.

O. T. SCHULTZ.

DISTRIBUTION OF IMMUNE BODIES IN THE PROTEIN FRACTIONS OF THE SERUM. A. A. SCHMIDT and KLARA TULJTSCHINSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:311, 1933.

The proteins of the serum were precipitated with a saturated solution of ammonium sulphate. Agglutinins of the typhoid bacillus were present predominantly in the euglobulin fraction and to a lesser degree in the pseudoglobulin fraction. Antisheep hemolysins of rabbit serums and complement-fixing antibodies of human syphilitic serums were found almost entirely in the euglobulin fraction. The albumin fraction did not contain any of the mentioned antibodies.

I. DAVIDSOHN.

CHEMICAL EXAMINATION OF THE SPLEEN IN GAUCHER'S DISEASE. H. MAI, *Ztschr. f. Kinderh.* **55**:12, 1933.

The spleen from a person with Gaucher's disease was examined chemically and found to contain from 8 to 9 per cent kersin. The sterin and phosphatide content was less than normal. A normal spleen examined by the same chemical method showed no noteworthy amount of cerebrosides. The chemical examination confirmed the clinical diagnosis of Gaucher's disease.

JACOB KLEIN.

## Microbiology and Parasitology

BLOOD CULTURES IN CHRONIC ARTHRITIS. E. F. TRAUT, *J. Infect. Dis.* **52**:230, 1933.

Seventy-one per cent of thirty-eight patients with chronic arthritis yielded primarily bacillary or diplococcic forms in forty-one instances and coccoid forms in chains in three instances. These bacteria had the characteristics of enterococci. On isolation the earliest colonies resembled the G colonies of Hadley. The essentials of successful blood cultures in chronic arthritis are prolonged cultivation, faultless technic, suitable culture mediums and the recognition of pleomorphism and dissociation.

AUTHOR'S SUMMARY.

HEMOGLOBINOPHILIC BACILLI FROM INFANTILE MENINGITIS. B. E. EDDY, J. Infect. Dis. **52**:242, 1933.

The gram-negative hemoglobinophilic bacilli of infantile meningitis grew most luxuriantly on blood agar when first isolated from the spinal fluid. Apparently some constituent of the spinal fluid is especially favorable for their growth. All strains required blood in the medium even after two years' artificial cultivation. All strains examined produced indol, and a medium to which tryptophan was added was found to enhance growth greatly. The sugar fermentations of the hemoglobinophilic bacilli confirmed the observation of Rivers and Kohn with the exception of one strain. All of the strains of hemoglobinophilic bacilli studied were uniformly pathogenic for young white rats and nonpathogenic for chickens.

AUTHOR'S SUMMARY.

PLEOMORPHIC ORGANISM SHOWING RELATIONSHIPS BETWEEN STAPHYLOCOCCI AND ACTINOMYCETES. M. V. NOVAK and A. T. HENRICI, J. Infect. Dis. **52**: 253, 1933.

A yellow staphylococcus was recovered from triturated, Berkefeld-filtered cultures of an actinomycete after incubation for five weeks at room temperature. This organism was culturally and morphologically identical with staphylococci on ordinary routine mediums. On dextrose agar, rods and branching filaments developed from the coccoid forms. Filaments or rods reverted to cocci when transferred again to plain agar. Smooth and rough colonies were obtained from cultures of the yellow staphylococcus. Rods and coccoid forms appeared in both smooth and rough colonies. A G type culture was obtained from aged broth cultures of the organism. The elements in the G culture ranged in size from very minute to normal. These G forms demonstrated no filamentous properties and were strictly bacterial in nature. It is suggested that the observations support the theory that the staphylococci are related to the actinomycetes.

AUTHORS' SUMMARY.

BACTERIOLOGIC INVESTIGATION OF THE BLOOD IN RHEUMATIC FEVER. B. R. CALLOW, J. Infect. Dis. **52**:279, 1933.

Diplostreptococci (alpha type) and pleomorphic bacilli may be recovered repeatedly from the blood of patients with rheumatic fever and certain diseases mostly of the upper respiratory tract. These organisms apparently represent stages in the life cycle of the same organism. A specific etiologic relationship between these organisms and rheumatic fever is questioned.

AUTHOR'S SUMMARY.

DIFFERENTIATION OF BOVINE AND PORCINE STRAINS OF BRUCELLA ABORTUS BASED ON DISSOCIATION. B. S. HENRY, J. Infect. Dis. **52**:403, 1933.

A method for the differentiation of strains of *Brucella abortus* of bovine from those of porcine origin, based on the size relations of the R and S colonies in the two strains, is presented. In the porcine strains the R colonies are smaller than the S colonies, whereas the reverse is true in the bovine strains.

AUTHOR'S SUMMARY.

THE BIOLOGY OF THE FERMENTING SARCINAE. J. SMIT, J. Path. & Bact. **36**: 455, 1933.

The occurrence of different fermenting sarcinae (brought together into the genus *Zymosarcina*) in natural materials has been studied, and the morphology and biology of these forms are dealt with. The sugar metabolism of *Zymosarcina ventriculi* and *Zymosarcina maxima* has been examined. Comparison of their vitality in pure cultures with that under natural conditions leads to the view that the coccal form of sarcinae represents a sensitive form of the organism which dies

out in a few days, and grows out irreversibly from a stable form, in which sarcinae universally occur and keep alive for long periods in natural materials like soil, sand and bran of cereals. The external shape of this form has not up to now been determined.

AUTHOR'S SUMMARY.

THE PHOTODYNAMIC ACTION OF METHYLENE BLUE ON BACTERIOPHAGE. J. R. PERDRAU and C. TODD, *Proc. Roy. Soc., London, s. B* **112**:277, 1933.

The concentration of 1:100,000 of methylthionine chloride, U. S. P. (methylene blue) in a broth filtrate of a bacteriophage specific for *Staphylococcus krueger* produced the complete inactivation of the lytic principle on exposure to light for eight minutes. This phenomenon did not take place in the absence of oxygen. Other strains of bacteriophage which were examined were less susceptible. Higher concentrations of methylthionine chloride were less effective, probably because of the absorption and loss of light in the upper layers of fluid. The inactivating action of the light employed was limited to the wavelengths absorbed by a solution of methylthionine chloride. Attempts to reactivate the bacteriophage by reduction were not successful. A protecting action by the corresponding living organism was observed. Killed organisms had no such effect. The protective action was not strictly specific as it was exhibited by certain heterologous bacteria.

L. E. SHINN.

THE PHOTODYNAMIC ACTION OF METHYLENE BLUE ON CERTAIN VIRUSES. J. R. PERDRAU and C. TODD, *Proc. Roy. Soc., London, s. B* **112**:288, 1933.

The viruses of vaccinia, herpes, fowl plague, thortor ill, Borna disease, Fujinami's tumor and canine distemper, in cell-free fluids, were inactivated by a concentration of 1:100,000 of methylthionine chloride, U. S. P. (methylene blue) on suitable illumination for a few minutes. The viruses of foot and mouth disease and of infectious ectromelia were more resistant. As with bacteriophage, oxygen was essential for the inactivation. The viruses of herpes, Borna disease and fowl plague were protected by the presence of living cells from the infected animal. Only a slight protection was observed in the case of vaccinia and no protection in the case of thortor ill. The development of the specific lesions of vaccinia and herpes could be prevented by suitable treatment with methylthionine chloride and light within from twelve to twenty-four hours after inoculation.

L. E. SHINN.

TUBERCULOUS BACILLEMIA. JOHN CRIBBIN, *Tubercle* **14**:163, 1933.

From 190 tubes inoculated with blood according to Löwenstein's method, and in addition on Holm and Schwabacher's mediums, obtained from 17 cases of advanced pulmonary tuberculosis, no positive results were obtained. The patients had advanced cases with repeatedly positive sputum and well marked and severe toxic symptoms. Most of the patients died within from one to five months from the commencement of the investigations; only four are still alive.

H. J. CORPER.

TUBERCULOUS BACTEREMIA. J. TROISIER, T. DE SANCTIS MONALDI, R. CATTAN and MME. KOURILSKY, *Ann. Inst. Pasteur* **49**:614, 1932.

With the Loewenstein technic for the cultivation of tubercle bacilli from the blood stream, organisms were recovered "in the course of nontuberculous infections," viz., a streptococcic septicemia, an acute pneumonia that was cured without sequelae and a meningococcic meningitis. The organisms were of the human type, thereby excluding avian organisms from the eggs used in the culture medium. It seemed probable that these organisms arose from latent foci, which might mean either apathogenicity or the origin of active processes.

M. S. MARSHALL.

SYPHILIS IN GUINEA-PIGS. J. VAN HAELST, *Ann. Inst. Pasteur* **49**:778, 1932.

Inoculation of guinea-pigs with material rich in spirochetes was successful, lesions appearing in four weeks and lasting from two to eight weeks. The lesions, especially after several passages, consisted of papules, nodules and ulcerations, rich in organisms and keratitic, which may have been metastatic. The author prefers for the present to consider syphilis as a localized disease in this animal.

FROM AUTHOR'S CONCLUSIONS.

CATARACT IN RABBITS EXPOSED TO HERPES. S. NICOLAU and MME. L. KOPCIEWSKA, *Ann. Inst. Pasteur* **50**:117, 1933.

"In résumé, four cases of cataract were observed in rabbits during the course of immunization or in rabbits already immunized against herpes virus, of which one appeared to illustrate the fortuitous localization of the organism in the crystalline lens, since the opacification in this case appeared at the peak of the disease. In the other three cases, on the contrary, the cataract could probably be interpreted as trophic trouble at a distance, consequent on changes in the nerves. However that may be, we have thought it of interest to report these cases which may perhaps suggest the origin of certain human cataracts, in which a neurotropic virus is involved in provoking nerve lesions."

SPONTANEOUS TUBERCULOSIS IN ANIMALS. A. CALMETTE, *Ann. Inst. Pasteur* **50**:148, 1933.

Spontaneous tuberculosis in rabbits and guinea-pigs has been observed from Koch's time to date. A review of the literature, combined with the extensive work with BCG vaccine, has given the author many data for observing the practical aspects of this problem and the dangers in experimental work with tuberculosis. Virtually all stages of tuberculosis appear to occur frequently, some so delayed that they frequently are not observed. This fact explains the individual differences observed in animals housed together, all tuberculous, and some showing a type of infection much different from others. There is no fundamental difference between the guinea-pig, the rabbit and other susceptible animals and man with regard to this infection.

M. S. MARSHALL.

BLOOD CULTURE OF TUBERCLE BACILLI. E. LÖWENSTEIN, *Ann. Inst. Pasteur* **50**:161, 1933.

A method of blood culture, used extensively by the author in Vienna, consists of a rather complete concentration method, starting with citrated blood, and finally inoculation of a medium consisting of eggs, asparagine solution, potatoes, congo red and malachite green. Organisms were cultivated from various forms of tuberculosis, from acute and chronic polyarthritis, from early cases of chorea and even from patients with dementia praecox.

M. S. MARSHALL.

THE TUBERCULOUS ULTRAVIRUS. G. SANARELLI and A. ALESSANDRINI, *Ann. Inst. Pasteur* **50**:167, 1933.

Some of the general conclusions by the authors from an exhaustive study of the so-called ultravirus by the collodion sac method are: The ultravirus passed collodion filters in vivo and in vitro. The pathogenicity of filtrates made either way was too weak to cause nodule formation. Cultivation was accomplished, though not easily, by using the pulp of organs of animals of the second or third passage. The peritoneal cavity of animals infected through a sac revealed bacterial forms, "tuberculous protogens." From these forms, on proper medium, developed typical tubercle bacilli, but cultures were frequently sterile. Such recovered organisms were feebly pathogenic, but by repeated growth recovery of virulence was rapid.

Inoculation of guinea-pigs with protogens resulted in characteristic, chronic, fatal processes, with inflammatory or caseogenous anatomic changes, absence of visible tuberculous granulomas, and the frequent presence of acid-fast rods. The idea of the existence of a complex cycle in the life of the organism was considered to be confirmed.

M. S. MARSHALL.

NEW STUDIES IN EXPERIMENTAL SYPHILIS. C. LEVADITI, A. VAISMAN, M. R. SCHOEN and J. G. MEZGER, *Ann. Inst. Pasteur* **50**:222, 1933.

In an extensive report three phases of the subject are considered. Regarding the question of whether *Spirochaeta pallida* is only one visible phase of a complex cycle, it is stated: "Although new investigations are necessary to complete our knowledge of the evolutionary cycle of the syphilitic virus, one may admit as very plausible, without being definitely demonstrated, the concept of the existence of an infravisible phase in the evolution of *Spirochaeta pallida*." This chapter serves as a preface to a study of neurosyphilis in monkeys and rabbits, with careful histologic study, in which evidence was found that cyclic phases of strains, some more neurotropic than others, occurred in the parenchyma of the nerve. The third point at issue concerns the possible avirulence of the "pale spirochete" in the spirochete stage: "The preceding observations, added to those recently reported by Jahnelt, Prigge and Rothermundt, make us hesitate to subscribe to the concept of the avirulence of the pale spirochete, ingenious and original as it may be."

M. S. MARSHALL.

THE DOG AND THE "BUTTON FEVER" VIRUS. PAUL DURAND, *Arch. Inst. Pasteur de Tunis* **21**:239, 1932.

The dog was studied as a potential reservoir for this typhus-like disease, apparently transmitted to man by the dog tick. In some instances experimental infection was induced, with a positive Weil-Felix reaction. Dogs showed little or no reaction, but successful transfer to man (fever therapy in dementia praecox) was accomplished by subcutaneous injection of dog's blood.

M. S. MARSHALL.

THE DESIGNATION "FIÈVRE BOUTONNEUSE." CHARLES NICOLLE, *Arch. Inst. Pasteur de Tunis* **21**:347, 1932.

"At different times, my lamented friend E. Conseil and I have been concerned with the employment of new and varied terms to designate the disease described for the first time by A. Conor and A. Bruch, in Tunis. I have demanded, moreover, that the term exanthematic fever should not be used to designate a particular disease, but the family of diseases of which the historic exanthematic typhus is the oldest and best known, 'button fever' being classed in this same family. The First Congress of Mediterranean Hygiene, meeting at Marseilles on Sept. 20 to 25, 1932, which counted among its members present most of those who in recent years have advanced our knowledge of 'button fever,' J. Pieri, Et. Burnet, Paul Durand, D. and J. Olmer, G. Blanc and J. Caminopetros, Plazy, Mercandier and Pirot, Pecori, Combiesco, etc., has adopted the following resolution:

"It [the Congress] is of the opinion that the denomination 'button fever' (*fièvre boutonneuse*), already employed, should be retained to designate the disease, described for the first time by Conor and Bruch in Tunis and discovered at various points around the Mediterranean basin, especially in Marseilles by Olmer, in Italy and in other countries. The term 'exanthematic fever' should be reserved for the group of diseases of which exanthematic typhus is the type and of which button fever is a part.

"Thus is definitively established the excellent term under which the Tunis doctors recorded, in 1910, this new disease of the group of exanthematic fevers."

APPENDICITIS AND ANGINA. L. ASCHOFF, Beitr. z. path. Anat. u. z. allg. Path. **87**:481, 1931.

Aschoff studied the relation of angina to acute appendicitis by bacteriologic investigation of the bacteria responsible for each condition. In about 70 per cent of cases of acute appendicitis the etiologic organisms are the anhemolytic streptococci *Enterococcus* A and B. In angina the predominating organism was found to be a hemolyzing streptococcus (60 per cent). Common to both was the presence in a small percentage of cases of the pneumococcus. Aschoff emphasizes that for each site the foregoing organisms are normal inhabitants, and that an inflammatory attack is due to a local increase in virulence. A sequential relation between angina and appendicitis in view of the disparity of the bacteriology is denied.

W. S. BOIKAN.

CONGENITAL TUBERCULOSIS OF THE LUNG DUE TO ASPIRATION OF AMNIOTIC FLUID. M. SIEGEL, Beitr. z. path. Anat. u. z. allg. Path. **90**:503, 1932.

A premature infant, whose mother died of pulmonary tuberculosis two days after the birth of the child, died at the age of 19 days. Symptoms of pneumonia developed four days before death. Both lungs were the seat of a widespread tuberculous bronchiolitis and caseous pneumonia. The process is considered a primary lesion that resulted from the aspiration of amniotic fluid containing tubercle bacilli.

O. T. SCHULTZ.

EXPERIMENTAL TUBERCULOSIS OF THE PANCREAS. A. VON VERES, Beitr. z. path. Anat. u. z. allg. Path. **90**:673, 1933.

The reported rarity of tuberculous involvement of the pancreas in generalized human tuberculosis led to the experiments here reported. Intraperitoneal, subcutaneous or direct intrapancreatic inoculation of guinea-pigs was followed by tuberculosis of the pancreas. The author concludes that pancreatic tissue offers no especial resistance to tuberculous infection and that more careful histologic examination of the pancreas in human tuberculosis would reveal a greater frequency of involvement of this organ than has been reported.

O. T. SCHULTZ.

THE CULTIVATION OF THE VIRUS OF INGUINAL LYMPHOGRANULOMA. KURT MEYER and H. E. ANDERS, Klin. Wchnschr. **11**:318, 1932.

Cultures were prepared from human lymphogranuloma exudate, the procedure of Maitland being used, and the virus was propagated in passage. The liquid culture was injected into guinea-pigs in the inguinal region. In half of the animals, changes occurred in the inguinal glands and in one instance in an associated iliac gland which corresponded to the changes in human glands. If identification of an infectious disease is acceptable by means of histologic methods, then the cultivation and propagation of the virus of lymphogranuloma of the inguinal glands appears possible.

DAVID O. ROSBASH.

DETECTION OF TUBERCLE BACILLI IN CIRCULATING BLOOD. A. AXEN, Klin. Wchnschr. **11**:1949, 1932.

No tubercle bacilli could be isolated from the blood of diseased guinea-pigs or human beings, or from tonsillar tissue. The discrepancies between the results of this experiment and those obtained by Löwenstein could not be attributed to differences in technic or in the culture medium employed.

D. O. ROSBASH.

BACILLEMIA AND BACILLURIA IN TUBERCULOSIS. H. DEIST, Klin. Wchnschr. **12**:26, 1933.

From 287 patients, including 213 women with all forms and stages of tuberculosis, 18 children with different localizations of the disease, 36 mature patients

with extrapulmonary tuberculosis, and 30 nontuberculous patients, the author obtained 4 positive cultures from the blood of 3 patients with tuberculosis, employing Löwenstein's method. Culturing urine daily for twenty-five days from 31 patients having neither vesical nor renal tuberculosis resulted in 12 positive and 19 negative results.

D. O. ROSBACH.

HEMATOGENOUS INFECTION OF THE TONSILS. C. KRAUSPE, *Virchows Arch. f. path. Anat.* **287**:139, 1932.

Previously reported results of experimental work had led the author to conclude that the tonsils may be involved by way of the blood stream and that the resulting involvement is often not to be readily distinguished from such as occurs by way of the exposed surface of the tonsil. The present communication is based on the histologic examination, in serial sections, of the tonsils of eleven children and fourteen adults who died of a variety of acute infectious diseases. The tonsils of twenty-five children whose death was not due to acute infection were also examined, but somewhat less thoroughly. In most of the cases of infection, especially in children, the tonsils revealed inflammatory reactions that the author interprets as the result of hematogenous localization. The earliest stages were noted in and about the arterioles of the follicles and in the subepithelial capillaries. Necrosis and abscesses were frequently observed. The bacteria could usually be demonstrated in the lesions. Streptococci were the most important invading organisms in children. In both children and adults, localization of bacteria in the subepithelial capillaries led to inflammatory foci not to be distinguished from primary crypt infections. Involvement of the tonsils was seen in one case of typhoid and one of bacterial glomerulonephritis.

O. T. SCHULTZ.

VALUE OF ANIMAL INOCULATION IN THE DIAGNOSIS OF UNDULANT FEVER. H. LOTZE, *Virchows Arch. f. path. Anat.* **287**:162, 1932.

As an aid in the diagnosis of infections by *Bacillus abortus*, Lotze strongly recommends animal inoculation. For each inoculation he uses two full-grown guinea-pigs, one male and one female. The nonagglutination of the organism by the blood of the animals should be determined prior to their use. The material to be inoculated is injected subcutaneously at the bedside of the patient. The character of the material used is not stated; presumably it is blood. The diagnosis may be established within from three to seven days after inoculation by performing an agglutination test with blood withdrawn from the inoculated animals by heart puncture. The temperature curve of the animals is diagnostic at the latest by the fourth week after inoculation. The diagnosis may be further established in the fifth week by the macroscopic lesions of the inoculated animals.

O. T. SCHULTZ.

PARALYTIC DEMENTIA AS A SPIROCHETOSIS. W. K. NELEZKY, *Virchows Arch. f. path. Anat.* **288**:346, 1933.

The brains of twenty unselected and untreated persons with progressive paralysis were subjected to microscopic study with special reference to the presence of spirochetes and to the numerical relationship of the latter to the clinical course of the disease. The organisms were demonstrated in fifteen (75 per cent) of the brains. Exclusion of brains with arteriosclerotic softening and of the brains of those who died of acute infection with high fever increased the positive percentage to 100. The number of typical and degenerated spirochetes bore a direct relation to fatal seizures and to the duration between the latter and the time of death. The seizures of paralysis are the result of periodic increase in the number of spirochetes in the cerebral cortex. During such periods of increased multiplication, the organisms may appear in the circulating blood. Death due to marasmus may be preceded by an increase in the number of organisms without any definite change in the characteristic symptoms of the



disease. When death followed a seizure, many of the spirochetes were partly lysed and many had been phagocytosed. Lysis predominated over phagocytosis. The latter process was by the cells of the mesoglia, which the author considers a part of the reticulo-endothelial system.

O. T. SCHULTZ.

PHAGOCYTIC ACTIVITY OF CAPILLARY ENDOTHELIUM. W. KLOSTERMEYER, *Virchows Arch. f. path. Anat.* **288**:703, 1933.

Although some observers have ascribed to ordinary capillary endothelium the capacity for vital storage, the weight of opinion would limit this phenomenon to the reticulo-endothelium in the narrower sense. The difference in the phagocytic activity of the two kinds of endothelium may be due to differences in surface tension or in electric charge. To investigate the latter possibility, the electric charge of bacteria was altered. But when such bacteria were injected into mice they were not more readily taken up by the capillary endothelium of the lung than were untreated bacteria. If the animals were sensitized with either a specific or a nonspecific antigen, the capillary endothelium of the lungs took up bacteria as readily as did the reticulo-endothelial system.

O. T. SCHULTZ.

PURULENT MENINGITIS DUE TO BACILLUS ENTERITIDIS. P. RIEPER, *Virchows Arch. f. path. Anat.* **289**:301, 1933.

Meningeal symptoms developed in a 3½ month old nursling seven days before death. *Bacillus enteritidis* was grown in pure culture from the spinal fluid during life. The total duration of the illness was twenty-six days; it began with a furuncle of the buttock, which was followed by furuncles on other parts of the body. Five previously reported cases of meningitis due to *B. enteritidis* are summarized; all occurred in young nurslings.

O. T. SCHULTZ.

AN UNUSUAL BACTERIUM IN ABSCESES OF THE BRAIN. WILLY BENDER, *Zentralbl. f. Bakt. (Abt. 1)* **122**:469, 1931.

Four patients with abscesses of the brain revealed an unusual type of bacterium as the causative agent. The micro-organism was found in pure culture either in the cerebrospinal fluid or in the abscesses, showed marked variations from coccus to rod forms, did not ferment the usual sugars and was gram-negative and non-motile. The author suggests the name *Bacterium alternans*.

PAUL R. CANNON.

THE FATE OF PARENTERALLY INTRODUCED BACTERIOPHAGE IN THE BODY. P. SMIRNOW and M. GOLDIN, *Zentralbl. f. Bakt. (Abt. 1)* **122**:512, 1931.

Bacteriophage against the dysentery bacilli of Shiga and of Flexner was injected subcutaneously or into the testes of guinea-pigs and its presence in the various organs determined. It disappeared from the blood, liver, kidneys, lungs, brain, testes and intestine within from one to three days following subcutaneous injection, but persisted in the lymph nodes and spleen for from thirteen to seventeen days. When injected directly into the testes it persisted there for two weeks. When the bacteriophage, however, was injected subcutaneously into guinea-pigs previously immunized against it, it could be recovered within twenty-four hours only from the liver, spleen and lymph nodes and had disappeared from these organs at the end of forty-eight hours.

PAUL R. CANNON.

### Immunology

THE RESPIRATION OF PHAGOCYTOSIS. C. W. BALDRIDGE and R. W. GERARD, *Am. J. Physiol.* **103**:235, 1933.

By means of the Warburg manometer, the respiration of leukocytes during phagocytosis was studied. A constant increase in oxygen consumption was observed

during this activity, the rise beginning at once, reaching a maximum value of about twice that of the inactive state in about fifteen minutes and ending in from ninety to one hundred and fifty minutes. The main burst of extra respiration lasted from ten to fifteen minutes, after which the maintained level was relatively little above the control value. The authors believe that this phase may represent the liberation of extra energy in digestion, the earlier and greater rise being that of engulfment.

H. E. EGGERS.

AN UNUSUAL BLOOD GROUP. M. M. WILHELM and E. E. OSGOOD, Arch. Int. Med. 52:133, 1933.

A subgroup ( $B_1$ ) of group B is described which is characterized by a serum that agglutinates 33 per cent of group B cells and 47 per cent of group O cells and by cells which are agglutinated by 43 per cent of group B serums. From absorption experiments the characteristics of this serum may be explained by the presence of a single extra agglutinin in the group  $B_1$  serum and of a corresponding single agglutinogen occurring in some of the cells in each of the three groups (A, B and O) tested. Previously reported subgroups are summarized in a table. Precautions necessary to prevent accidents are outlined. AUTHORS' SUMMARY.

ANTIPOLIOMYELITIC SERUM FROM MONKEYS. FREDERICK EBERSON, J. Immunol. 24:433, 1933.

Macacus rhesus monkeys were apparently immunized against the virus of poliomyelitis by a series of subcutaneous injections of a culture of an organism cultivated from Berkefeld N filtrates of poliomyelitis virus. The protection conferred was measured by the neutralizing power of their serums for poliomyelitis virus. In two instances the resistance of the monkeys to intracerebral injection of the virus was also tested. The serums neutralized the poliomyelitis virus in vitro completely or in part. The two intracerebral inoculations showed that the monkeys had become resistant to infection by this route. During the course of immunization with the living cultures one animal contracted typical poliomyelitis. In an earlier series of experiments three monkeys showed symptoms and signs of typical abortive infection. The cultures used were in the twenty-fourth subplanting and represented a dilution of the original virus material of approximately  $2 \times 10^{-38}$ . The results are discussed with reference to a possible survival of any hypothetical adsorbed virus and with reference to the viability of the virus as compared with the cultures at incubator temperature. The experiments, it is believed, confirm further the previously reported observations (*J. Lab. & Clin. Med.* 18:565, 1933) on the possible relationship of this organism to the poliomyelitis virus.

AUTHOR'S SUMMARY.

AGGLUTINATION IN VARICELLA. C. RUSSELL AMIES, Lancet 1:1015, 1933.

The findings of Aragao and of Paschen that elementary bodies are present in the fluid of the vesicles of varicella have been amply confirmed. A method of preparing purified suspensions of these bodies is described. Such suspensions are specifically agglutinated by the serum of patients convalescent from varicella. The results obtained in a series of sixty-one agglutination tests are recorded. The constant presence of the elementary bodies in the fluid of the early vesicles and the fact that they are specifically agglutinated by the homologous antiserum are regarded as strong evidence in favor of the view that these represent the infecting agents of varicella.

AUTHOR'S SUMMARY.

SPECIFIC LESIONS OF THE KIDNEY AND LIVER INDUCED BY CYTOTOXIC SERUMS. M. MASUGI, Beitr. z. path. Anat. u. z. allg. Path. 91:82, 1933.

For his experimental investigation Masugi used the rat, whose small size makes it possible to use relatively large doses of antiserum. He ascribes the

negative and contradictory findings of the many investigators who have preceded him in this field to the fact that insufficiently large doses of serum were used when rabbits and dogs were employed as the experimental animals. The nephrotoxic and hepatotoxic antisera were prepared in the rabbit, and a relatively high degree of organ specificity was obtained. In the kidney the primary damage produced by the nephrotoxic antiserum was to the glomerular capillaries and the afferent arterioles. In the liver the hepatotoxic antiserum manifested its primary effects on the vessels of the peripheral zone of the lobule, the small vessels of the interlobular tissue and the small branches of the hepatic artery. The vascular reaction was similar to that recently described by a number of observers in local hyperergic inflammation. The parenchymatous alterations are secondary to the changes in the vessels. The resulting lesions are like those of the kidney in human glomerulonephritis and those of the liver in eclampsia, from which the author concludes that allergy, or antibody-antigen reaction in its wide sense, is the underlying factor in the pathogenesis of these two human diseases.

O. T. SCHULTZ.

POSTMORTEM CHANGES IN THE SPINAL FLUID. S. SÜMEGI, Frankfurt. Ztschr. f. Path. **44**:283, 1932.

Normal antibodies appeared in the cerebrospinal fluid about twenty-four hours after death. At first complement alone could be demonstrated, but later, about thirty-four hours after death, normal hemolysin was also present. The appearance of the normal antibodies is correlated with the increase in the amount of protein in the cerebrospinal fluid after death and also with the shift to the left of the precipitation zone of the colloid curves.

AUTHOR'S SUMMARY.

REACTIONS OF TISSUE IN EXPERIMENTALLY SENSITIZED RABBITS. K. APITZ, Virchows Arch. f. path. Anat. **289**:46, 1933.

A series of twenty-four rabbits was sensitized with horse serum. At various intervals after sensitization the rabbits received a subcutaneous injection of the antigen, and notations were made of the resulting local Arthus reaction. The animals then received a massive intravenous dose of the antigen. Seven died of acute shock, five more died after prolonged symptoms of shock, and twelve recovered and were killed at varying periods. The heart, liver, spleen and blood vessels were subjected to histologic study. The reactions described are in part degenerative and in part inflammatory. The degenerative reactions consisted of focal albuminoid degeneration and necrosis, followed by the deposition of calcium, which was noted especially in the liver and heart. Inflammatory proliferative and lymphocytic infiltrative reactions were noted, especially in the myocardium of the right side of the heart and in the blood vessels. Further mesenchymal reactions were large cell transformation of the spleen and the development of monocytic and myeloid cells in the liver. The development of the changes described is influenced by non-specific factors. Whether the mesenchymal changes are to be interpreted as a defense reaction the author was not able to determine from his experiments.

O. T. SCHULTZ.

LYSIS OF TUBERCLE BACILLI IN SENSITIZED RESISTANT SPECIES OF ANIMALS. PHILIPP SPANIER, Zentralbl. f. Bakt. (Abt. 1) **121**:451, 1931.

The author maintains that the leukocytes of animals which are resistant to tuberculosis (the dog, horse and rat), when mixed with tubercle bacilli and kept for five days at 37 C., cause more granular degeneration of the bacilli than do the leukocytes of susceptible animals (the guinea-pig, the rabbit and man). He assumes, therefore, that the leukocytes of the resistant animals contain more tuberculolytic substance and are better prepared to liberate undenatured antigen from the tubercle bacilli. Dogs were infected intraperitoneally with a bovine strain of a virulent tubercle bacillus and were reinfected subcutaneously in a sterile abscess produced

by the injection of oil of turpentine with from 50 to 100 mg. of living tubercle bacilli. Acid-fast stains, made twenty-four hours later, showed many granular forms of tubercle bacilli in the pus from the abscess. Cultures of this material were negative, and guinea-pigs which received injections of it remained healthy. Similar material from the abscess was diluted with a sterile salt solution and placed in sterile flasks, at 37 C., and 50 mg. of living tubercle bacilli was placed in it. After one week the tubercle bacilli contained many granules, and many were no longer acid-fast. After from two to two and one-half months, cultures of this material were sterile. Observations on pus from the abscesses of normal control dogs showed a persistence of tubercle bacilli in the mixture. The injection of the autolysate from the previously infected dogs into tuberculous guinea-pigs caused a general tuberculin reaction. Similar material, autolyzed for two months, when injected into rabbits and guinea-pigs, caused no tuberculosis. The possibilities of using such autolysates in immunization are being studied and will be reported on later.

PAUL R. CANNON.

THE SHWARTZMAN PHENOMENON. H. GROSS, *Zentralbl. f. Bakt. (Abt. 1)* **122**: 96, 1931.

Gross has confirmed most of Schwartzman's observations concerning the Schwartzman reaction. He obtained the reaction with bacterial filtrates only and not with horse serum, broth or agar. The best results were obtained by injecting the "reacting" dose from twenty to twenty-four hours after the "preparatory" dose; no effect was produced with an incubation period of less than two hours or of more than thirty-six hours. Desensitization occurred if a bacterial filtrate was injected intravenously at the same time that the preparatory dose was injected. The reaction could not be elicited in guinea-pigs, and Gross was unable to obtain it in the lungs, eyes and joints of rabbits. He concludes that the reaction is one of hypersensitivity, but that it differs from anaphylaxis or the Arthus phenomenon.

PAUL R. CANNON.

RÔLE OF THE SKIN IN THE FORMATION OF AGGLUTININS. A. TRAWIŃSKI, *Zentralbl. f. Bakt. (Abt. 1)* **123**:336, 1932.

Portions of skin from rabbits highly immunized against paratyphoid-enteritis micro-organisms were transplanted into normal rabbits whose blood serums contained no agglutinins before the transplantation. Agglutinins soon appeared in the serum of the latter rabbits, the maximum titer being reached on the twelfth day. If the transplanted skin was injured by the induction of necrosis, the agglutinin titer of the blood serum dropped promptly, and if comparable amounts of skin from highly immunized rabbits were macerated and injected subcutaneously into normal rabbits, only traces of agglutinin appeared in the blood serum. The author concludes that the agglutinins are not transferred passively in the transplantations of skin, but that they are produced locally in the skin of the rabbit because of the large amount of histiocytic tissue in the skin.

PAUL R. CANNON.

RELATION BETWEEN AN ACID OR AN ALKALINE DIET AND IMMUNITY: A. M. BONANNO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:19, 1932.

The acid-base equilibrium of rabbits and guinea-pigs was changed by feeding them with calcium and ammonium chloride, with sodium bicarbonate and with sodium citrate. An acid diet was followed by a decrease in the bactericidal and complementary qualities of the blood. A change in the normal albumin-globulin ratio in the blood was noted, there being a rise in albumin and a drop in globulin. An alkaline diet was without effect. An acid diet impaired the ability to produce immune agglutinins and to resist infection and increased the severity of anaphylactic reactions. A modification of the diet in either acid or alkaline reactions led to a marked lowering of resistance to intraperitoneal infection with tubercle bacilli.

I. DAVIDSOHN.

APPLICATION OF RECENT PRECIPITATION REACTIONS FOR THE DEMONSTRATION OF SYPHILIS IN RABBITS. F. E. HAAG and W. LEVEN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:25, 1932.

The Kahn test gave uniformly negative results with diluted serums (1:10) of normal healthy rabbits. The reactions were positive in a considerable number of cases when undiluted serum was used or when the Kahn citochol or the Meinicke test was employed. Latent syphilis could not be detected by means of any of these tests. In the presence of an active infection the Kahn citochol test was the only one that produced a considerable number of positive reactions, but in view of the positive results in healthy rabbits no definite conclusions could be drawn. No differences were observed between the serum reactions of animals infected with syphilis and those of rabbits infected with *frambesia tropica*. The results contradict the report of Taishin Saito (*Ztschr. f. Hyg. u. Infektionskr.* **110**:603, 1929), who claimed to have found in the Kahn test with diluted serum a means of diagnosing syphilis in the rabbit. The Kahn citochol reaction and particularly the second modification of the Mueller "*Ballung*" reaction were found very well suited to the diagnosis of syphilis in rabbits, but only after precipitation of globulins in the serum with diluted hydrochloric acid by the method of Sachs and Georgi. Triple and double dilutions had to be employed instead of the original tenfold dilution.

I. DAVIDSOHN.

SEROLOGIC SPECIFICITY OF PLACENTAL TISSUE. G. F. DE GAETANI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:43, 1932.

Serums of rabbits immunized with an aqueous suspension of human placental tissue reacted with placental extracts and with human red blood cells. The material used for immunization was not free from red blood cells. No attention was paid to the blood group of the persons whose placentas were employed. Some organ specificity was present, as demonstrated by proper absorption experiments. A few of the serums also reacted with the placenta of the ox. From the fact that the albumin fraction of the antisera was shown to contain the bulk of the antibodies, Gaetani concludes that the antibodies in the serum were of the Forssman type, but he admits that their source is probably in the red blood cells not removed from the placental tissue. No reaction took place with boiled placental tissue. Immune serums obtained with boiled placental tissue contained species-specific lipid antibodies.

I. DAVIDSOHN.

ORAL IMMUNIZATION WITH VIRULENT STAPHYLOCOCCI AND STREPTOCOCCI. HANS WINZELER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:60, 1932.

Guinea-pigs weighing from 230 to 280 Gm. were fed living or killed cultures of highly virulent strains of *Staphylococcus aureus* or *Streptococcus haemolyticus*. Some of the animals were treated with ox bile, a sodium salt of a bile acid or sodium benzoate previous to being given the bacterial suspension. When living bacteria were used, active immunity against subsequent parenteral injections of multiple lethal doses of *Staph. aureus* was obtained in seventeen of twenty-five animals. Dead bacteria only slightly increased the natural resistance. The immunization with streptococci was not successful. The preliminary administration of bile or bile salts was not found necessary.

I. DAVIDSOHN.

SEROLOGIC ANALYSIS OF EGG WHITE. M. SHARMA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:79, 1932.

Immune serums produced in rabbits with boiled egg white reacted best in complement-fixation tests with the homologous antigen heated to 100 and to 80 C.; the fixation was either insignificant or absent with egg white heated to below 70 C. If the quantity of the antibody was increased, complement was fixed even with the raw antigen. A similar specificity prevailed in the immune serums produced

with raw egg white, which did not react with antigen heated above 80 C. Egg white heated at 70 C. occupied a middle position, being able, more than any other, to produce antiserum which under proper conditions reacted both with unheated and with boiled antigens, though here also the optimum fixation occurred with the homologous antigen. The absorption of complement-fixing antibodies in an immune serum obtained with boiled egg white was also best with the homologous antigen. Extracts of egg white prepared with 50 per cent alcohol fixed complement with some boiled egg white antisera; other alcoholic extracts failed to react.

I. DAVIDSOHN.

## Tumors

EXPERIMENTAL EPITHELIAL GROWTHS IN THE LUNG AFTER INSUFFLATION OF TAR. L. M. SCHABAD, *Ztschr. f. Krebsforsch.* **38**:154, 1932.

Schabad studied the effects of a single intratracheal insufflation of coal tar on the lungs of guinea-pigs, the animals being kept under observation up to fourteen months. There was an epithelial overgrowth of adenomatous type, with a stroma of inflammatory connective tissue; the growth could be differentiated from adenoma by the lack of elastic tissue in the stroma. In regard to the sources of the epithelium, the author concludes that, in part, it is of bronchial origin; but the occurrence of similar lesions in the extreme periphery of the lung, far from direct bronchial communication, leads him to the belief that in part it may come from the alveolar linings, contrary to the usually accepted current view that these are not of epithelial character. He infers, with apparent justice, that some of the alleged experimental cancers of this organ reported after the use of tar are really the type of lesion he obtained. In these growths, there was no ground for assuming a malignant character; they never penetrated the pleura, nor were metastases ever observed. Indeed, Schabad feels called on to explain why cancer did not occur; he lays little stress on the fact that guinea-pigs are notoriously immune to the cancerigenic action of tar, and states that in his belief this property is lost soon after application to tissue, and that thereafter tar behaves as a relatively inert oil, so that repeated applications are necessary to exert a cancerigenic effect.

H. E. EGGERS.

PHOSPHATASE IN TUMORS. S. EDLBACHER and WALDEMAR KUTSCHER, *Ztschr. f. physiol. Chem.* **207**:1, 1932.

The activity of the nucleotidase in the liver and in tumors can be increased through acetone extraction of the tissues. The acetone extract contains an inhibitor of the nucleotidase. Cysteine, glutathione and hydrocyanic acid, as well as copper ions, inhibit the nucleotidase. The natural inhibitor is possibly identical with glutathione. The activation of nucleotidase in minced liver by hydrocyanic acid is explained by the high heavy metal content of this organ. It is suggested that the nucleotidase and arginase are heavy metal ferments. While the tumor hexophosphatase is as strongly inhibited by cysteine, glutathione and hydrocyanic acid as the nucleotidase, the liver hexophosphatase is only half as much inhibited by hydrocyanic acid as the nucleotidase and is only weakly or is not inhibited by cysteine and glutathione.

WILHELM C. HUEPER.

HEREDITARY NEUROFIBROMA AND DIFFUSE MENINGEAL GLIOMATOSIS. F. HARBITZ, *Norsk mag. f. lægevidensk.* **93**:841, 1932.

In a case of neurofibroma and diffuse meningeal gliomatosis in a boy who died at the age of 9, Harbitz saw a threefold interest. The hereditary basis was marked; the child was the sixth of a family of nine children, of whom the five oldest had multiple neurofibromas. Recklinghausen's disease in the mother, then 19, was described by Harbitz in 1908. In addition to the peripheral neurofibroma, the patient had gliomatosis in the central nervous system, with the starting point

probably in the cauda equina, and these two tumors are regarded as an outcome of the same constitutional factor, the case thus connecting neurofibroma in the peripheral nerves with gliomatosis in the central nervous system. The diffuse extent of the masses in the leptomeninges of the spinal cord and the brain was so pronounced that the cord and medulla seemed to be cast in tumors.

### Medicolegal Pathology

DEMONSTRATION OF CARBON MONOXIDE IN THE EXHUMED CADAVER. STANISLAW LAGUNA, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **21**:512, 1933.

Reports in the literature indicate that carbon monoxide has been successfully demonstrated in the disinterred cadaver from fourteen to one hundred and twenty-two days after death. The author had occasion to exhume a body two hundred and ten days after death. In spite of the fact that the tissues were putrefying, a definite bright pink color was noted in the muscles, in some of the organs and in the pleural and peritoneal transudates. Spectroscopic examination of these transudates demonstrated the absorption band for carbon monoxide hemoglobin.

JACOB KLEIN.

TRAUMATIC INTRAPERITONEAL RUPTURE OF THE URINARY BLADDER. STANISLAW LAGUNA, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22**:14, 1933.

A drunken woman, hospitalized on suspicion of poisoning, died four days after admission. At autopsy the abdominal cavity contained 2,500 cc. of a clear, straw-colored fluid. The peritoneum was hyperemic and covered with a fibrinopurulent exudate, particularly on the wall of the bladder, which showed a midline tear 5 cm. long. The history indicated that the patient had fallen while dancing and that her partner had accidentally stepped on the lower part of her abdomen.

JACOB KLEIN.

FLUORIDE POISONING. M. FLAMM, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22**:21, 1933.

A 22 year old workman in a brewery accidentally swallowed some 30 per cent hydrofluosilicic acid used as a disinfectant and was immediately seized with cramps, vomiting and diarrhea. He died four days later, after the development of convulsions, cyanosis and stupor. At autopsy there were destruction of the epithelium in the pharynx and esophagus, congestion of the larynx and trachea, marked edema of the lungs, bleeding and necrosis of the gastric mucosa, inflammation and punctate hemorrhages in the jejunum, acute toxic hemorrhagic nephritis and cerebral hyperemia and edema. The use of fluorides should be restricted and more strictly supervised.

JACOB KLEIN.

GROUP SPECIFICITY OF SPERMATOZOA. A. SCHMIDT and H. ECK, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22**:43, 1933.

By means of agglutinin absorption tests it is possible to determine the blood group from spots of semen on linen, even after from two to three years. The spermatozoa belong to the same blood group as the person's blood. This is of practical medicolegal significance.

JACOB KLEIN.

SUBGROUPS  $A_1$  AND  $A_2$  IN INVESTIGATIONS OF PATERNITY. ERIK WOLFF and BENGT JONSSON, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22**:65, 1933.

A determination of subgroups  $A_1$  and  $A_2$  is readily made by the glass slide test with a standard 1 per cent erythrocyte suspension, the readings being both macroscopic and microscopic. In children  $A_2$  cannot be determined before the

age of 6 months. Studies in 883 cases of doubtful paternity confirm the four gene hypothesis of blood group heredity. A table has been compiled which gives the blood groups of the mother and child and indicates the groups which eliminate a person as the possible father. Moreover, given a father with a certain blood group, that of the mother and child may be determined. The forensic importance of subgroups is emphasized, especially groups A<sub>1</sub> and A<sub>2</sub> and M N.

JACOB KLEIN.

SPONTANEOUS RUPTURE OF AN AORTA WITH MUCOID DEGENERATION OF THE MEDIA. FRITZ HELLNER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22:86**, 1933.

A 49 year old man collapsed while lifting a heavy barrel and died shortly afterward. There were hemopericardium, cardiac hypertrophy, dilatation of the ascending aorta and a tear in the posterior wall of the aorta above the posterior valve. Microscopically there were mucoid degeneration of the media and destruction of the elastica. The author discusses the various causes of spontaneous rupture of the aorta as described in the literature.

JACOB KLEIN.

TECHNIC OF DETERMINATION OF M AND N GROUPS IN BLOOD STAINS. A. LAUER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22:86**, 1933.

Landsteiner's technic based on the principle of absorption was used. The blood stain, washed out with distilled water, was mixed with a 2 per cent suspension of standard erythrocytes on glass slides which had been cleaned with a 10 per cent solution of liquid petrolatum in xylene. Using this technic, the authors were able to recognize M and N groups in forty specimens of dried blood from one week to three months old. The method was also successfully used in two medicolegal cases.

JACOB KLEIN.

DETERMINATION OF BLOOD GROUPS BY EXAMINATION OF HUMAN FECES. H. HODYO, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **22:95**, 1933.

Group-characteristic substances are found in body fluids, secretions and excretions. The author mixed from 10 to 20 Gm. of feces with twice as much physiologic solution of sodium chloride or distilled water and filtered the mixture through several layers of gauze. The filtrate was dried on a water bath at from 50 to 70 C. The resulting powder was tested by the absorption technic. Blood group characteristics were found in the normal stool which agreed with those shown by the control tests on the person's blood. Sometimes the reaction was distorted by the presence of hemolysins, agglutinins and inhibiting substances in the stool.

JACOB KLEIN.

DEATH DURING BATHING. FRITZ BERNSTEIN, *München. med. Wchnschr.* **79:1889**, 1932.

Grassl has reported attacks of illness during bathing which may be attributed to cold allergy. This condition is manifested by urticaria, hemoglobinuria, asthma, collapse, lowering of the blood pressure, leukocytosis and, in particularly susceptible persons, unconsciousness. The reaction occurs usually from five to fifteen minutes after exposure to cold. Doubtless some cases of drowning are due to cold allergy in persons who have previously shown evidence of hypersensitiveness to cold. A simple test for determining the existence of such an idiosyncrasy is to place a piece of ice on the healthy skin for from one to two minutes; after from five to fifteen minutes a wheal forms in the susceptible person.

JACOB KLEIN.



DEATH DURING BATHING. S. J. THANNHAUSER, München. med. Wchnschr. **79**: 1890, 1932.

A 46 year old athletic physician noticed erythema and urticaria of the palms and soles after bathing in cold water. Wheals were noted on other parts of the body on exposure to cold water. After a shower there was redness of the entire body, followed by a greenish-white discoloration and a feeling of chilliness which disappeared after half an hour. While bathing in a mountain lake after vigorous exercise he observed erythema, pruritus and a feeling of oppression in the chest. He immediately left the water and sank into a chair. Although he was not unconscious, he was weak and unable to move. Vigorous colonic peristalsis soon developed; the skin was greenish, and the pulse was slow. There was a feeling of intense cold over the entire body. He recovered after one hour. In the evening, while eating, he experienced a sudden feeling of cold and loss of appetite, but there were no changes in the skin. This was relieved by drinking alcohol. Cold urticaria and resulting shock represent an exaggeration of the normal heat regulation; perhaps a histamine substance is liberated. Drowning may readily result from the adynamic reaction.

JACOB KLEIN.

BULLET EMBOLUS OF THE PULMONARY ARTERY. R. PALTAUF, Wien. klin. Wchnschr. **46**:602, 1933.

A 21 year old man was shot in the third intercostal space 3 cm. from the midline. Death occurred from hemopericardium seven days afterward. The track of the bullet led through the upper lobe of the left lung and the pericardium at the origin of the pulmonary artery, which showed no wound of exit. A 6.35 mm. steel jacket bullet was found in the branch of the pulmonary artery to the lower lobe of the right lung, where it had caused pressure necrosis with infarction of the lung. The pleuritis resulting from the infarct provoked coughing, which opened the wound in the pulmonary artery and caused death from hemopericardium. This is one of the few instances in which a bullet has been transported by the circulation.

## Technical

STAINING OF OLIGODENDROGLIA AND OF MICROGLIA IN CELLOIDIN SECTIONS. ARTHUR WEIL and HAROLD A. DAVENPORT, Arch. Neurol. & Psychiat. **30**:175, 1933.

The authors give a modification of Stern's method of staining oligodendroglia and microglia in celloidin sections. For the staining of microglia, the sections are washed in distilled water, are put for ten or twenty seconds into an ammonia-silver nitrate solution, from which they are transferred to a solution of formaldehyde, and after three changes of water, are dehydrated and mounted in Canada balsam.

Practically the same method is used for staining oligodendroglia, with slight modifications, for which the original article should be consulted. The appended photomicrographs bring out well the advantages of the modified silver staining methods as given by Weil and Davenport.

GEORGE B. HASSIN.

IMPORTANCE OF ADEQUATE REDUCTION OF PEPTONE IN THE PREPARATION OF MEDIA FOR THE PNEUMOCOCCUS AND OTHER ORGANISMS. H. D. WRIGHT, J. Path. & Bact. **37**:257, 1933.

Difficulty in preparing broth suitable for the cultivation of pneumococci is largely due to incomplete reduction of peptone. This may readily be overcome by adding peptone to the broth before any heat is applied and so exposing it to the powerful reducing action of meat or meat infusion during the steaming process. Huntton's method of preparing broth owes its advantages to the fact that it provides for adequate reduction of the peptone. Other features in this method, especially filtration through glass wool, have been found to be of minor importance.

The presence of oxidized peptone in broth renders it relatively unsuitable for the cultivation of many aerobes and for that of *Clostridium tetani*. This inhibitory effect must be taken into account in experiments relating to accessory factors in growth and the oxidation-reduction potential.

AUTHOR'S SUMMARY.

STAINING OF THOROTRAST IN TISSUE SECTIONS. L. PRÜSENER, Beitr. z. path. Anat. u. z. allg. Path. **91**:439, 1933.

The Best carmine method for glycogen stains thorotrast in tissue sections. A staining time of from ten to fifteen minutes is necessary. A concentrated alcoholic solution of chromotrope 2R, applied for from five to ten minutes, also stains thorotrast. A hematoxylin nuclear stain may be combined with either of these methods.

O. T. SCHULTZ.

NEW METHODS OF PRECIPITATION AND "BALL" REACTIONS FOR THE DEMONSTRATION OF SYPHILIS. JULIUS KISS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:195, 1932.

Kiss published, in 1930, a book entitled: "The Technic and Theory of the Examination of the Serum for Syphilis." In the present article, he summarizes the results of his studies since the publication of the book. Kiss advocates the use of antigens of known chemical composition. He employs a mixture of lecithin and cephalin prepared from alcoholic and ether extracts of the heart. Cephalin is the more sensitive of the two substances. It reacts with unheated serum. Various modifications of the Kahn precipitation test and of the Mueller *Ballung* test are offered. In the former, a more stable solution of the antigen is produced by removal of the alcohol from the antigen-salt solution mixture. In the Mueller test, the modified antigen contains mainly cephalin, with some admixture of lecithin. Unheated serum and different concentrations of sodium chloride are among the innovations. The *Ballung* test is, according to Kiss, the most sensitive of all methods. However, he emphasizes the danger of relying on precipitation tests or on the *Ballung* test only, and advocates strongly their employment together with a complement-fixation test.

I. DAVIDSOHN.

EXAMINATION OF EXTRACTS FOR THE WASSERMANN REACTION BY MEANS OF NEPHELOMETRY. J. ADAMSKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:247, 1932.

The ability of varying quantities of the extracts and of their fractions to prevent the precipitation of cholesterol was estimated with the apparatus of Moll. The animal species from which the heart muscle was obtained and the method of preparation influenced the protective ability of the extract. By combining certain extracts, the protective effect could be improved. Adamski gained the impression that extracts from hearts of animals killed during the summer and fall had greater protective ability than the heart extracts of animals killed during the winter or early spring. Such dependence on the time of the year may have some relation to the feeding.

I. DAVIDSOHN.

PRACTICAL VALUE OF THE NEW MODIFICATION OF THE CITOCHOL REACTION. L. S. SCHIRWINDT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:294, 1932.

The new modification of the citochol reaction was published by Sachs and Witebsky in 1931. The changes concern mainly the dilution of the antigenic extract, with a resulting marked increase of sensitiveness. Schirwindt made parallel tests on 1,219 serums, employing the old citochol reaction, the Wassermann reaction and the Kahn test along with the new modification. The last test gave 8.4 per cent more positive reactions for persons known to have syphilis, and 10.7 per cent

more for persons with latent syphilis. To overcome the possibility of nonspecific positive reactions, which were very scarce, the parallel employment of the old modification is suggested. The use of a 3 per cent dilution of sodium chloride and the shaking by hand, if a shaking apparatus is not available, are preferable to the other procedures, such as using lower dilutions of sodium chloride and leaving the test materials at room temperature for from four to six hours. In experimental syphilis of rabbits, the modification of the citochol reaction proved by far the most sensitive, the next in sensitiveness being the Kahn reaction. Schirwindt compares the new modification with the "presumptive procedure" of Kahn.

I. DAVIDSOHN.

COMPARISON OF EXTRACTS OF SACHS-WITEBSKY WITH OWN EXTRACTS FOR THE CITOCHOL REACTION. S. L. SCHIRWINDT and A. V. ALEXEJEVA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:353, 1932.

Extracts prepared by the authors proved as efficient as those procured from Sachs' laboratory. That is considered an advantage as it removes the necessity of depending on one laboratory. The citochol reaction with cerebrospinal fluid showed a distinct lack of sensitiveness, but the test appears to have been greatly improved in this respect by the new modification of the antigen.

I. DAVIDSOHN.

MODIFICATION OF THE REACTION OF TSIENT-YUNG-TSÜ WITH INACTIVATED SERUM FOR THE DIAGNOSIS OF SYPHILIS. S. L. SCHIRWINDT and M. B. FEDOROWA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:359, 1932.

Tsien-Yung-Tsü utilizes shaking instead of the primary incubation in his technic of the complement-fixation test. He recently published a modification of the test in which inactive serum is used, and fresh rabbit serum is employed as the bearer of antisheep hemolysin and of complement. Schirwindt and Fedorowa preserve rabbit serum with 1 per cent of boric acid. In a series of 1,002 comparative tests, they found the modified method more sensitive than the Wassermann, the Kahn and the citochol reactions. They have not encountered nonspecific positive reactions.

I. DAVIDSOHN.

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, Nov. 13, 1933*

E. H. HATTON, *President, in the Chair*

### A RARE DENTAL ANOMALY ("DENS IN DENTE"). RUDOLF KRONFELD.

The dental anomaly usually described as *dens in dente*, or tooth inclusion, clinically appears as an enlarged or malformed anterior tooth the pulp chamber of which contains a small toothlike structure. This condition seems to be rare. Only about twelve cases have been reported in the literature. Most of these were said to be a twin formation whereby one tooth (parasite) developed within another (autosite). The arrangement of the hard tissues—enamel and dentin—in the inner tooth is inverse to that of the outer tooth. Thus the enamel of the outer tooth is on the outside and the dentin is on the inside, while the enamel of the inner tooth is inside of the dentin. By serial histologic sections through a tooth with this anomaly, it was possible to demonstrate that it is not a twin formation but a malformation of the germ of only one tooth, namely an invagination of one portion of the developing crown into the other. This explains not only the inverse arrangement of enamel and dentin in the inner invaginated portion but also the connective tissue and bone occasionally found in the central cavity within the *dens in dente*. The bone inclusion is periodontal in origin and has become pinched off and included in the center of the invaginated portion of the crown.

### DISCUSSION

E. F. HIRSCH: The anomalous structure suggests the possibility of a fusion of two incisor-like teeth in mirror-image juxtaposition in which the structures of the root on the approximated surfaces either failed to develop or disappeared, while those of the crown persisted and fused.

### INFECTIOUS CIRRHOSIS: REPORT OF A CASE. JAMES D. STEWART.

A white woman, aged 39, was well until three and a half years prior to death, when generalized pruritus was noted. This soon was complicated by attacks of icterus, gray stools, dark urine, moderate loss of weight and a tendency to bleed easily, but there was no pain or gastro-intestinal symptoms. These symptoms persisted through her illness. The first examination, fifteen months after the onset, disclosed an icteric, nervous person with a blood pressure of 110 systolic and 74 diastolic and a pulse rate of 72. The liver was enlarged and palpable on deep inspiration. Roentgen examinations revealed a normal stomach and duodenum, nonvisualization of the gallbladder and no stones. The results of the usual laboratory examinations were not significant except for an absence of free acid in the gastric contents after Ewald, histamine and alcohol meals. An exploratory operation disclosed nothing abnormal except enlarged and fibrosed periportal nodes. The condition persisted for two more years and became progressively worse. The liver gradually enlarged, and the spleen became palpable. No ascites developed. Hepatic function was adequate until a few months before death. An examination of excised skin eliminated hemochromatosis. When the patient entered the Billings

Hospital for the last time one month before her death she presented marked jaundice, a prolonged coagulation time, impaired hepatic function, anemia, a reticulocyte count of 6 per cent and the usual fragility of the red cells. Bile was obtained by duodenal tube. A cholecystoduodenostomy was performed and no further changes were noted. The patient's condition was satisfactory for three days, then a hemorrhage into the wound occurred, and her condition became progressively worse. Death followed ten days after the operation.

Necropsy disclosed an enlarged liver and spleen, no ascites, mild localized fibrinous and hemorrhagic peritonitis, a functional cholecystoduodenostomy, enlarged periportal lymph nodes, a patent biliary tract, a slightly dilated thin-walled gall-bladder with a few small calcium carbonate and pigment concretions, bilateral hematomas of the ovary, a hematoma of the upper pole of the right kidney, absence of the right suprarenal gland, slight chronic fibrous mitral endocarditis and edema of the lower lobes of the lungs. The liver weighed 3,150 Gm. and was finely nodular. It showed slightly increased resistance and had a deep yellowish-green, firm, glistening parenchyma with a uniformly accentuated, translucent stroma. The spleen had a dark red pulp and weighed 700 Gm. The kidneys were jaundiced and large. The gastro-intestinal tract revealed no changes.

Histologically the liver had an increased perilobular and peripheral intralobular stroma. There were dense infiltrations of the portal areas by small round cells, mononuclear leukocytes and a few polymorphonuclear leukocytes and macrophages. The biliary epithelium was destroyed, and there were accumulations of bile pigment in small bile ducts and adjacent lobules. Stains and microchemical tests failed to demonstrate bacteria or iron.

The prolonged icterus, the large liver and spleen, the absence of ascites, the clay stools, the inflammation of the finer bile ducts, the necrosis of the biliary epithelium and the marked infiltration and perilobular and intralobular fibrosis correspond with the changes of infectious cirrhosis described by Mallory in 1911 and 1932 and by McMahon in 1931. The changes are similar to the clinical and pathologic disorder described by Hanot in his thesis published in Paris in 1875.

#### DEMONSTRATION OF MICRO-ORGANISMS IN EPIDEMIC ENCEPHALITIS (ST. LOUIS EPIDEMIC). ARTHUR WEIL.

Through the cooperation of the department of pathology of Washington University, St. Louis, I was able to study representative blocks of tissue from the brains of eight persons who died from encephalitis in the recent epidemic. In sections of each brain treated with paraffin and stained with cresyl violet or methylenethionine chloride, U. S. P. (methylene blue), diplococci were demonstrated. They were from 0.6 to 1 micron in diameter, isolated or accumulated in small colonies or arranged in short or in long chains. The diplococci within the subarachnoid spaces were somewhat larger than those in the brain tissue. They were not demonstrated, however, within the perivascular cellular exudate or within the foci of proliferated glia. These facts, together with the absence of an inflammatory reaction in the regions where these diplococci were found, suggest that they were able to multiply only after the defense reaction of the body had broken down, and that the process was either agonal or postmortem. The diplococci were not found as the result of accidental contamination, but must have been present at the moment of death, because they were found in the centers of blocks of tissue fixed with formaldehyde as well as in the peripheral portions.

These observations are reported because the simultaneous appearance of one and the same type of micro-organism in colonies and chains in each of a number of brains received from different hospitals is unusual. Furthermore I was impressed by the large number of "hyperkinetic" types of encephalitis among the cases occurring in St. Louis. Von Economo has advanced the theory that perhaps in this type there is a mixed infection as compared with the classic ophthalmoplegic-lethargic type, which he considered a pure virus infection.

## DISCUSSION

E. F. HIRSCH: Were there leukocytic exudates or reactive changes in the tissue about the foci of bacteria?

N. PAUL HUDSON: It is not generally agreed what the etiology of the epidemic encephalitis of von Economo is. Numerous investigators believe that it is as yet unknown, and bacteria are not commonly accepted as the primary cause. The significance of the diplococci, as shown by Dr. Weil, is uncertain owing to the failure of other workers accurately to reproduce the disease with them. There is a difference of opinion as to the actual nature of the epidemic that occurred in St. Louis, whether it was of the von Economo or of a different type.

If one looks into the preliminary reports (Muckenfuss) of the attempts to isolate an infectious agent from the cases occurring in St. Louis, one finds that bacteria that could be considered significant have not been recovered. On the other hand it appears that injection of material from human brains into monkeys and mice has transmitted an infectious agent that is not cultivable on bacteriologic mediums, is not visible, is filtrable, and, hence, falls in the general group of viruses.

Under these circumstances, the primary significance of the diplococci in the brain is doubtful. They were apparently present before or at death, and I should like to ask Dr. Weil what the intervals of time and the conditions were between death and the fixation of tissue.

L. E. DAY: Epidemics of encephalitis have occurred in horses, and from the brain tissues of these animals many different kinds of bacteria have been cultured, but none has proved to be the infectious agent.

A. WEIL: The usual procedures for preserving and storing brain tissues were followed in the material I studied. It was furnished to me in blocks hardened in formaldehyde. My study was entirely morphologic.

## MYOCARDIAC CHANGES IN HYPERTENSION. VICTOR LEVINE.

This article will appear in full in a later issue of the ARCHIVES.

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NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Oct. 26, 1933*

PAUL KLEMPERER, *President, in the Chair*

## AN INSTANCE OF PLASMOCYTOMA WITHIN A TUBERCULOUS CAVITY. DAVID PERLA.

A man, aged 68, stated that he had had a productive cough for the past fifteen years. In January, 1932, his cough became more severe and spasmodic, and the sputum was blood-streaked for several weeks. He had severe anorexia and slight loss of weight. At that time he showed signs of consolidation of the right apex, with marked dulness, bronchial breathing and crepitant râles. He had a slight fever for a few days, and the sputum was found to contain tubercle bacilli. Roentgenograms showed a large dense shadow in the right upper lobe. There were numerous nodular infiltrations throughout both lungs; these were interpreted as metastatic nodules in addition to tuberculosis. A mass was felt in the right lower quadrant of the abdomen, and roentgen examination suggested the presence of a malignant condition in the region of the cecum. The patient was fairly comfortable during the following eight months. Then dizzy spells, difficulty in walking and defects in speech and memory developed. In January, 1933, there developed spastic right hemiplegia and complete motor aphasia. He died one month after admission to the hospital.

Autopsy disclosed a primary carcinoma of the cecum with metastases to both lungs and to the brain and thyroid, and generalized arteriosclerosis with slight coronary sclerosis. There was evidence of chronic bilateral pulmonary tuberculosis. A large fleshy tumor was found in the right upper lobe, apparently situated within a tuberculous cavity. In the central portion of the tumor was a firm nodule 2 cm. in diameter composed of dense, yellowish tissue containing several areas of calcification and possibly osseous formation.

The tumor was composed of plasma cells throughout. The other nodules in the lung were definitely adenocarcinomatous metastases from the cecal carcinoma. Of interest is the occurrence of this unusual plasmacytoma within a tuberculous cavity in a patient who had a primary carcinoma of the cecum with metastases to the lungs.

#### DISCUSSION

ALFRED PLAUT: Have the bones been irradiated with the roentgen rays in this case?

DAVID PERLA: No, we did not get permission for a complete autopsy. Has any one seen a case like this of a plasma cell tumor in the lung? I have tried to find one.

#### A CASE OF COOLEY'S ANEMIA. DAVID PERLA.

A boy, aged 9 years, with a previous history of measles at 5 and tonsillectomy at 6 years of age, was admitted to the hospital with weakness, edema of the lower extremities and progressive anemia. Three years prior to admission a large spleen had been noted. Shortly thereafter edema of the legs was observed, but this cleared up spontaneously in two months. The patient was said to have been anemic practically since infancy. In addition to marked pallor, he presented a subicteric tint of the skin, the mongolian facies and the rachitic rosary. There was prominence of the frontal and parietal bones. There was a sanguinopurulent discharge from the left ear. The heart was enlarged to the left. A precordial systolic murmur was heard. The spleen and liver were markedly enlarged.

The hemoglobin was 30 per cent; the red blood cell count, 2,200,000; the white blood cell count, 10,500, with a differential smear showing 2 per cent myeloblasts and 3 per cent myelocytes. Normoblasts and megaloblasts were present. Erythroblasts were seen in small numbers. There were marked poikilocytosis and anisocytosis.

A roentgenogram of the skull showed marked thickening of all the bones, with thinning of the cortex and a granular medulla. All the long bones showed thin cortices.

A diagnosis of Cooley's anemia was made.

The patient had a continuous fever up to 103 F. In spite of extensive liver, gastric and iron therapy with numerous transfusions, the anemia could not be improved, the red cell count remaining around 2,000,000. During the rises in temperature there was no evidence of any hemoclastic crisis, the urine showing no evidence of hemoglobin.

About one year after his first admission, following a transfusion, the patient died with the symptoms of dyspnea and cyanosis.

At autopsy a severe anemia was evident. The liver weighed 1,420 Gm., and the spleen, 725 Gm. There was a marked hemosiderosis of the mesenteric and retroperitoneal lymph nodes, with hyperplasia of the hemolymph glands and osteoporosis of the bones. The calvarium was thickened and measured 9 mm. in the occipital and frontal regions. The increase in thickness was due to a thickened medulla which was traversed by numerous fine trabeculations. The inner and outer plates each measured less than 1 mm. The vertebral medulla was chocolate brown.

Microscopic sections of the liver, pancreas, kidneys, mucosa of the stomach, and lymph nodes gave positive reactions for iron. Periportal accumulations of lymphocytes and plasma cells were seen. The spleen showed a moderate hyper-

plasia of reticulum cells; the sinusoids were congested; the endothelial elements were prominent and contained a brownish pigment that did not stain for iron. The malpighian follicles were atrophic. Scattered throughout the spleen were numerous eosinophilic myelocytes. The bone marrow was highly cellular, showing primarily myelocytes, erythroblastic elements and many groups of foam cells. Many of these contained ingested red cells.

## DISCUSSION

PAUL KLEMPERER: Of what race was the child?

DAVID PERLA: Italian. The picture you see in the bone marrow is present in sickle cell anemia and in congenital hemolytic icterus, but these three congenital anemias leading to rapid hyperplasia of the bone marrow are the only diseases which produce that picture.

PAUL KLEMPERER: I have seen the same large foam cells in the spleen in Cooley's anemia.

DAVID PERLA: They did not give the usual lipid stain of the foam cells.

## HYPERTROPHY OF THE HEART DUE TO STORAGE OF GLYCOGEN. W. ANTROPOL, J. HEILBRUNN and L. R. TUCHMAN.

A case of van Gierke's disease is reported which is believed to be the first one in which necropsy was done to be recorded in the American literature. There was tremendous enlargement of the heart together with hepatonephromegaly in a boy aged 4½ months. The heart weighed 85 Gm. Microscopically there was a massive infiltration of the myocardial fibers with glycogen. The liver and kidneys were the site of a similar deposition. The presence of appreciable amounts of galactogen was ruled out by comparison of Best's carmine preparations with those stained by iodine. Chemical analysis of tissue placed in alcohol after one month's fixation in formaldehyde showed 3.57 per cent glycogen in the heart, 3.25 per cent in the liver and 4.34 per cent in the kidney (wet weight). A relationship to idiopathic hypertrophy of the heart and rhabdomyoma of the heart is suggested.

## DISCUSSION

DAVID PERLA: Is the hypertrophy of the heart due to the mechanical storage within these cells, or did you see actual hypertrophy of the nuclear elements?

LOUISE W. RAUH (by invitation): About one and a half years ago an Italian boy, aged 20 months, was seen at Mount Sinai Hospital. He was thought to have a disturbance in glycogen metabolism. He had been perfectly well until 8 months of age. At that time it was noticed that his abdomen seemed to be larger than normal, and for the next year it increased in size. The birth and development had been normal, and the family history was irrelevant.

The child was pale, but fairly well nourished; he was somewhat stunted in growth. He was mentally normal and alert. There was an odor of acetone to his breath. The abdomen was large, the liver extending to below the umbilicus. The spleen could not be felt. There was no ascites. The remainder of the examination revealed no abnormalities.

On many occasions the urine showed acetone. There was a great increase in amylase, 19 units in a twenty-four hour specimen by the Elman method. There was no free glycogen, and no glycosuria after ingestion of 40 Gm. of dextrose.

The blood showed amylase 0.9 unit by the Elman method, from 2 to 6 units being normal. There was no free glycogen. The sugar content during periods of fasting varied between 40 and 65 mg. The curves for sugar tolerance were biphasic and showed prolonged elevation. There was a normal response of the blood sugar to epinephrine and to insulin. The urea was 17 mg., and the total cholesterol, 310, 415 and 395 mg. with an ester of 185, 120 and 290 mg. respectively. The lecithin was 325 mg.; the total fat, 2,300 mg. The calcium and phosphorus were normal. The icteric index was 6; the van den Bergh direct and indirect



tests were negative. The tests for hepatic function showed galactose and bromsulphalein to be normal. The tuberculin and Wassermann tests were negative. The cutaneous test for *Echinococcus* was negative. The blood count was normal; the Best stain of the blood smears showed no deposit of glycogen in the leukocytes.

Epinephrine therapy was attempted with subcutaneous injection of 0.2 cc. of a 1:1,000 solution twice a day for eight days. Following this there was no increase in the blood sugar during fasting nor any decrease in the size of the liver. Thyroid therapy was tried in an attempt to liberate the glycogen. It was begun with small doses, later increased to 0.12 Gm., administered three times daily, but after two weeks, because of loss in weight, it was discontinued. There was no change in the level of blood sugar during fasting or in the size of the liver.

The patient stayed in the hospital for three months, and grew 3 cm. in height and gained 1,800 Gm. The circumference of the abdomen increased 4 cm. At no time, in spite of the low blood sugar during fasting, were there any hypoglycemic reactions.

The patient is being followed; he is growing somewhat, but the size of the liver is steadily increasing.

PAUL KLEMPERER: Would it be impossible after four weeks to try to determine the presence or absence of enzymes in the organs?

CARL ZELSON: Dr. Schönheimer's conclusion that this was due to a disturbance in glycogen metabolism was based on the fact that in this condition after seven days the liver contained tremendous amounts of glycogen; in other words, in the normal liver glycogen is immediately broken down, and in this condition glycogen is not broken down in the liver. This is the main point in the interpretation of the disease. There is a disturbance in the glycolytic power within the cells of the liver.

LESTER R. TUCHMAN: The material was fixed in formaldehyde; therefore no studies of enzymes could be made. In spite of the fact that our figures are so high, large amounts of glycogen had undoubtedly been lost owing to the method of fixation. In one of the cases reported, half of the dry weight of the liver was glycogen, and in another case about 40 per cent of the dry weight was glycogen, so that apparently it can be stored in tremendous amounts. The clinical symptoms which Dr. Rauh described occurred in about half of the reported cases. The only common finding was the acetonuria. One of the patients had blood sugar as low as 15 mg. per hundred cubic centimeters without any hypoglycemic symptoms. One case is reported with no sugar in the blood ante mortem. Some patients were sensitive to insulin and some were not. Dr. Rauh's patient responded to epinephrine with a hyperglycemia; most did not. They all showed blood sugar that was low or "low normal." Kimmelstiel's patient with deposits of glycogen in the brain showed definite cerebral symptoms.

W. A. ANTOPOL: In answer to Dr. Perla's question, the nuclei are normal in size. If the fibers were reconstructed to aggregate solid units, they would probably approximate the normal dimensions. The storage of glycogen per se within the "hollowed-out" fibers caused the tremendous increase in size.

This condition is thought to be due to the persistence of the fetal type of glycogen metabolism in which the glycogen is mobilized with difficulty. Needham has shown the glycogen to be in a stable form just prior to birth. Diamanopoulos found further that more epinephrine is needed to mobilize glycogen in the new-born.

#### HODGKIN'S DISEASE IN THE LUNG. SYLVAN E. MOOLTEN.

The fundamental nature of Hodgkin's disease is still a problem; in respect to this problem the parenchyma of the lung, because of its unique construction, is a sort of arena in which can be observed the unfolding of the morphogenic characteristics of the disease in probably their simplest form. Most significant in this respect are the type and arrangement of the interstitial tissue, the epithelial content and behavior, and the air spaces themselves. The comparison of Hodgkin's

disease in the lung with certain infective granulomas of known etiology (e. g., tuberculosis and actinomycosis) on the one hand, and with tumors (including lymphosarcoma) on the other, tends to fortify the standpoint that Hodgkin's disease is primarily an inflammatory reaction of granulomatous character.

The material is drawn from a detailed study of eight cases embodying various aspects of the pulmonary lesion. In general two phases of the disease could be distinguished, the proliferative and the exudative. These were usually present simultaneously within the same area and even within the same lesion, the proportion of proliferative response to exudative response varying in individual cases. In general the proliferative type of lesion tended to display its most characteristic development within the interstitial structures of the lung, i. e., the walls and connective tissue sheaths of the bronchi and blood vessels, the interlobular septums and, above all, the interalveolar septums (i. e., the walls of the alveoli). The exudative phase occurred not only in these situations (where it was recognized with ease in some cases and with difficulty in others) but particularly within the air spaces of the alveoli. In no other tissue of the body is it possible to distinguish with such clarity the various degrees and kinds of the exudative response.

On the basis of personal observations of Hodgkin's disease as it implicates the lung together with findings reported in the literature certain conclusions are drawn. Hodgkin's disease is primarily an inflammatory condition, considered not only from the standpoint of its evolution within the organism as a whole but in particular from the standpoint of the type of response elicited within the lung.

What may be called the "pure lesion" as seen growing within the lumen of the alveolus unencumbered by any sort of stroma or other preexisting tissue is revealed not as the product of a specific proliferation of cells but rather as a specific mixture of cells (cells of histiocytic type, fibroblasts, cells immigrated from the blood).

Highly resistant structures such as the thick-walled arteries may become invaded by the surrounding infiltration, and on the other hand delicate structures such as the walls of the alveoli may likewise be infiltrated; in either case the result is not compression or destruction, but uniform infiltration with preservation of the underlying framework. This speaks for permeation by a virus or soluble toxin rather than for direct invasion by cells themselves as in a tumor. The proliferation of cells resulting in the specific mixture of cells is then primarily derived from the differentiation of the reacting preexisting autochthonous mesenchymal elements in response to the stimulus in loco.

On a similar basis it is possible to account for the abundant nonspecific inflammatory lesions. These consist of the mild "catarrhal" reaction of the alveoli, the more marked fibrinous exudative reaction and their subsequent end-stages. The catarrhal stage ends in the degeneration of the desquamated alveolar epithelial cells with liberation of their cholesterol in the form of the characteristic boat-shaped crystals. The fibrinous stage ends in some form of organization ("carnification") either by ordinary fibroblasts or by varying proportions of fibroblasts and cells specific to the granulomatous lesions ("pneumonia granulomatosa"). The ultimate fate of such lesions is transformation into ordinary scar tissue.

The alveolar epithelium does not play a mere passive part in the lesions but is apparently actively stimulated. In the exudative stage it undergoes rapid proliferation and desquamation in the form of large numbers of mononuclear or multinuclear phagocytic cells similar to those seen in many portions of tuberculous or actinomycotic lesions in the lung. These cells bear no more than a superficial resemblance to the Langhans giant cells or the Sternberg-Reed giant cells and are never seen to participate in the formation of the granuloma. In the proliferative and healing stages these cells form a continuous row of cuboid epithelium lining the infiltrated alveolar septums, often giving rise to the appearance of glands.

The distribution of the lesions within the lung conforms to that found in many bacterial infections of the lung. The proliferative phase occurs in two forms, peribronchial and pleurogenous, analogous to the two forms of interstitial pneumonia, peribronchial and pleurogenous (e. g., complicating grip or measles as an acute infection due to *Streptococcus haemolyticus*, or as part of tuberculosis or actinomy-

cosis). The exudative phase produces, in addition, a form of gelatinous lobar or lobular pneumonia.

From a synthesis of these findings the following classification of Hodgkin's disease of the lung is compiled: (1) granulomatous panbronchitis and bronchopneumonia, (2) exudative lobar and lobular pneumonia (gelatinous pneumonia, organizing pneumonia), (3) miliary, submiliary and multiple isolated nodular lesions (hematogenous, lymphogenous) and (4) granulomatous pleurogenous pneumonia (the primary form is rare, one case being reported; the secondary form is frequent, and is due to direct invasion of the pleura from the adjoining infiltrated mediastinum).

#### DISCUSSION

FRED W. STEWART: I think that this is a splendid contribution, and I watched Dr. Moolten's photographs with a great deal of interest because I have seen all these changes myself, and have come to much the same conclusion regarding their pathogenesis. I am glad to find some one who is willing to uphold the infectious nature of Hodgkin's disease despite all the recent negative bacteriologic findings by Twort and others, and especially after the suggestions in the last section of the Rose research on lymphadenoma, which tend to discourage the idea of Hodgkin's disease being an infectious granuloma. There is one type of involvement of the lung in Hodgkin's disease I should like to ask about. Within the last month or six weeks I saw a patient with typical Hodgkin's disease. He eventually died, showing essentially no lesions. He had a specific process in the spleen, but the manner of death was very unusual. A very intense interstitial emphysema suddenly developed, and at autopsy we found the usual signs of emphysema, an intense mediastinal emphysema, with spread of air to the tissues of the thoracic wall and neck. The lungs were tremendously distended. There was no point of perforation, and the only possible assumption was a rupture of the pulmonary alveoli. In the bronchi we found a complete cast of the bronchial tree, much as one finds in bronchial asthma. There was no history of previous attacks of bronchial asthma. The exudate in the bronchi was thick, of nearly the consistence of agar. There were a great many plasma cells and eosinophils, but nothing of the characteristic structure of Hodgkin's disease. On section the exudate in the bronchi, this heavy, thick mucinous cast, was full of eosinophils. That might have agreed well with bronchial asthma, but in the centers of most of these thick mucinous cores were areas of typical caseation. They had undergone caseous necrosis. In view of that, and of the fact that the patient had Hodgkin's disease and had never mentioned having had bronchial asthma I thought that this would have to be classed as one of the peculiar manifestations of Hodgkin's disease. Has Dr. Moolten ever seen anything like this?

SYLVAN E. MOOLTEN: In regard to the interesting type of bronchitis described by Dr. Stewart, the literature contains references to somewhat similar findings. I have not seen quite the same thing in the series I have studied, but in Hodgkin's disease lesions involving the bronchi have been known for many years and are well described in Ziegler's monograph of 1911. The gross appearance is often that of an ordinary catarrhal bronchitis, or it may be more like that of tuberculous bronchitis. Frequently there are stricture and stenosis of the bronchus and some times complete occlusion with atelectasis and secondary bronchiectasis. Bronchoscopy should prove useful in certain cases in which material is needed for biopsy in making a diagnosis. Characteristic findings by which one may suspect the diagnosis on gross inspection include the presence of certain areas of opacity or plaque formation in the mucous membrane, which are not quite the same as those seen in certain cases of carcinoma. At their periphery they tend to fade indefinitely into the surrounding mucosa. Furthermore, the lesions are usually distributed bilaterally and in a disseminated manner rather than grouped as a single lesion as in carcinoma. The presence of fibrinous exudate within the bronchi I should interpret as expressive of the more intense exudative reaction of the disease.

The question arises whether these acute exudative reactions, the fibrinous or catarrhal reactions, are specific or nonspecific. Morphologically they are unquestionably nonspecific. The question really concerns their specific or nonspecific causation, and that is an important point possibly admitting a certain amount of controversy. My own belief is that they are of specific causation, based on the findings in the lung itself. The distribution of such lesions does not correspond to the distribution of a bronchogenic lesion and does not follow the pathways of a bronchogenic dissemination such as might be produced by aspiration of infective material containing bacteria from the bronchi. The process suggests rather a diffusion of the virus itself from the central bronchial focus into the surrounding periphery in the parenchyma. The possibility that bacteria may secondarily contaminate the lesions in the lung has been mentioned by others and, incidentally, has been given as one of the reasons for the necroses often seen in these lesions. Actually, however, bacteria are rarely seen.

There are other points which would also speak for the diffusion of the hypothetical specific virus itself into these areas as a cause of the exudative reaction, such as the gradual transition from the nonspecific organizing pneumonia into the definitely specific lesions, in which one can see the gradual increase in the proportion of the specific cells over the nonspecific fibroblasts, forming a lesion in all respects identical with the first, except in the varying amount of the specific types of cells present.

The necrosis of the exudate in the bronchi mentioned by Dr. Stewart might speak for secondary infection. I have seen similar necrosis within alveolar exudate composed of masses of fibrin, with some leukocytes and alveolar phagocytes; the appearance at times is closely similar to the early lesion of tuberculosis. I do not know how to explain it. I do not know whether it is possible to explain it on the basis of the action of the specific virus itself causing necrosis of the exudate or of incidental bacterial contaminants. The stains I have made of such necrotic exudate have shown no organisms—neither ordinary bacteria nor tubercle bacilli.

#### AMYLOIDOSIS OF THE BONE MARROW, WITH REPORT OF A CASE. I. E. GERBER.

Amyloidosis of the bone marrow is a rare lesion. A number of instances of involvement of the media of small vessels of the bone marrow have been described. In addition a number of cases of primary amyloid tumors of the bone marrow have been reported. These cases are characterized by massive local accumulation of amyloid in one or several bones, with or without infiltrations in other organs. A third type is that of amyloid deposits in a true blastoma of the bone marrow. The multiple myelomas are the blastomas most frequently involved. No cases of diffuse involvement of the bone marrow have been reported hitherto.

The case reported is that of a white man, aged 44, who complained of generalized weakness and muscular pain. On examination hepatomegaly, signs of nephrosis and hypercholesteremia were found. Two years later hypertension, renal insufficiency and a collapse of the ninth dorsal and first lumbar vertebrae developed; the latter was thought to be the result of lipoid infiltration of the bones. Postmortem examination revealed generalized amyloidosis with diffuse amyloid infiltration of the bone marrow. No cause for the amyloidosis was found.

The amyloid in the organs stained with congo red and with the aniline dyes, but not with iodine. In the bone marrow the amyloid exhibited even more marked variations in staining and reacted only with the aniline dyes. There was marked destruction of the spongy bone without any tendency to the formation of new bone. Careful search failed to reveal any evidences of underlying disease of the bone marrow; no blastoma was found. As there was no tumor-like accumulation of the amyloid or underlying blastoma, this case represents a distinct type of amyloid disease of the bone marrow, i. e., diffuse amyloidosis of the bone marrow.

## DISCUSSION

DAVID PERLA: I have seen four instances of so-called primary amyloid disease in which no apparent etiologic factor could be found. The case presented is different from those which I have seen in that the bone marrow was not involved in any of them.

I think "typical amyloid disease" would be a better term than "primary amyloid disease," because one does not know what the nature of the amyloid deposition is, particularly if there is no destructive process in the body. In the instances which I have seen, the liver and spleen were not involved, and Lubarsch has stated that in atypical amyloid disease the amyloid was either slight or absent in the usual sites, and appeared in unusual sites. I have never seen a case like the one reported by Dr. Gerber, and I am very glad to have heard his presentation.

I. E. GERBER: I want to say a few words about the term "atypical." It was first employed by Lubarsch to denote an unusual localization of amyloid, in which the spleen and the liver, as Dr. Perla mentioned, are not involved. In going over the literature on amyloidosis, I have come across many instances in which there have been atypical localizations of amyloid, and in which the spleen and liver were involved, and these cases likewise showed all the other characteristics of amyloidosis, some of which were atypical, the staining reactions to the usual amyloid stains, for example, and the nodular deposits of amyloid. All of these may be found in cases of generalized amyloidosis—hence one must be careful in the use of the term "atypical." One must recognize the fact that some cases do tend to show atypical reactions, they do tend to spare the spleen and the liver, but they are in no way different from those of generalized amyloidosis. I do not want to enter into any new classification of the disease; the characteristics of an atypical amyloidosis are so typically found in generalized amyloidosis that I do not think it would be well to put in a third classification, unless there is a good reason for doing so.

A CASE OF CARCINOMA OF THE STOMACH TREATED AS PERNICIOUS ANEMIA:  
DURATION, OVER SIX YEARS. ANGELO M. SALA.

A woman, aged 65, was first seen in April, 1927, when she complained of weakness, poor appetite and a recent gradual loss of weight with increasing pallor of the skin. Her blood picture was as follows: erythrocytes, 1,500,000; hemoglobin, 40 per cent by the Dare method; color index, 1.3; leukocytes, 4,300; polymorphonuclear neutrophils, 50 per cent; lymphocytes, 46 per cent; large mononuclears, 3 per cent; eosinophils, 1 per cent. The coagulation and bleeding times were within the normal range. The stained smear showed poikilocytosis, anisocytosis, polychromatophilia and macrocytosis. There were occasional megaloblasts; normoblasts were not encountered. A reticulocyte count showed complete absence of reticulocytes in a count of several thousand erythrocytes. The report to the attending physician was that the blood picture strongly suggested addisonian anemia, but other diagnostic procedures were necessary to substantiate or exclude this diagnosis. The patient was given liver and hydrochloric acid with such marked improvement in two weeks that the physician was satisfied he was dealing with pernicious anemia, and continued to treat the patient accordingly. Her body weight, general clinical condition and hemoglobin were satisfactory during this time; on May 10, 1932, she weighed 143 pounds (65.86 Kg.), and her hemoglobin was 75 per cent. In August, the patient discovered a "lump" in her epigastrium. Surmising that her physician would recommend an operation, for which she considered herself a poor risk, she did not consult him until her regularly scheduled appointment in November. At this time a freely movable tumor the size of an orange was felt to the left of and above the umbilicus. There was no vomiting, but appetite had begun to fail. She was then sent to me, at the clinic of the New York City Cancer Institute, where the clinical diagnosis of carcinoma of the stomach was confirmed by the roentgenologist. At that time the hemoglobin was 35 per cent by the Dare method, and the red cell count was 1,000,000. The

morphology of the blood was about the same as in 1927; reticulocytes were absent. Operation and roentgen ray treatments were refused, and the patient gradually weakened and died at home on Aug. 5, 1933.

A postmortem examination limited to the abdomen revealed a bulky carcinoma of the stomach involving the distal two-thirds and the pyloric region. The tumor was necrotic, and there were metastases to the perigastric nodes and to the liver. There was no ascites. Histologically, the tumor was an adenocarcinoma, grade 2.

The question presents itself: Was this a case of gastric carcinoma arising in a patient with addisonian anemia, or did the patient have cancer of the stomach all along? I believe the latter has been the case; I have never seen gastric cancer develop in a patient with true pernicious anemia, and the duration is by no means against this probability. On the other hand, the case well illustrates the difficulty often encountered in basing a differential diagnosis between pernicious anemia and the anemia of gastric carcinoma on the examination of the blood alone. Pepper and Farley say in their "Practical Hematological Diagnosis": "Among the conditions which produce an anemia most closely simulating Addisonian anemia is carcinoma of the stomach or of the ascending colon. When such an anemia precedes local symptoms the diagnosis may be very much in doubt." It is my belief that the diagnosis of pernicious anemia is to be made largely by exclusion, and that it should remain provisional until all diagnostic procedures, of which examination of the blood is only one—and not necessarily the most important—are exhausted. Even in cases of apparently true primary anemia, in which the favorable response to the therapeutic test imparts a feeling of security, the possibility of a malignant condition of the gastro-intestinal tract and the desirability of roentgen studies of the digestive tract at not too long intervals need not be forgotten.

#### DISCUSSION

ALFRED PLAUT: I cannot say anything in relation to the pernicious anemia, but as far as the duration of the disease is concerned, it would not be as unusual as most people believe to have a carcinoma of the stomach with a course of six years or more. When gastro-enterologists go over the records of patients whom they have followed for years, they often find cases in which a long history of gastric disturbance ended with an operation for carcinoma or with an autopsy revealing this condition, so that in these cases one wonders whether the disease did not start long before the diagnosis was made or whether the patients had another condition of the stomach, and carcinoma developed later on. I have seen patients seeking treatment for from twelve to fifteen years concerning whom, incredible as it may seem, the most logical conclusion is that they probably had the same disease all the time. I remember one patient at the Memorial Hospital who, eight years after a biopsy which positively showed carcinoma, was still in the same condition, and the files of the Memorial Hospital contain records of cases which have been under observation longer than that.

ANGELO M. SALA: I entirely agree with Dr. Plaut. I believe that the patient had a carcinoma of the stomach from the beginning, and I present the case not because of its duration, but to call attention to a mistake that could and should have been avoided.

## Book Reviews

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**The Science of Radiology.** Authorized by the American Congress of Radiology. By twenty-six contributors. Edited by Otto Glasser, of the Cleveland Clinic Foundation. Price, \$4.50. Pp. 450, with 108 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1933.

One of the projects of the executive council of the recent American Congress of Radiology was the publication of a book which would record the outstanding developments in radiology from the time of Roentgen's discovery to the time of the congress. Beginning with the story of Roentgen and of Pierre and Marie Curie by Otto Glasser and an account of American pioneers in radiology by William A. Evans, there follows a consideration of roentgen physics, apparatus and tubes and recording mediums and screens. Dosimetry is discussed. Percy Brown summarizes in fifty pages the progress in roentgen diagnosis to date. Military roentgenology, roentgen cinematography and roentgen therapy are adequately described. Radium physics and dosimetry are considered by Failla and Quimby, while radium therapy is handled by Bowing and Fricke. George M. MacKee writes a valuable chapter on cutaneous roentgen and radium therapy. There are also chapters on protection, teaching, radiologic societies and literature, the nature of the cosmic rays and the Gurwitsch rays, and the foundations of therapy with ultraviolet, visible and infra-red radiations.

Chapters XVII and XVIII are of special interest to pathologists. Hermann U. Muller, professor of biology in the University of Texas, undertakes the consideration of the effects of roentgen rays on the hereditary material. Gene mutations and chromosome changes are produced by roentgen rays in germ cells of all types: in spermatozoa (whether irradiated while in the testis or after they have been received by the female), spermatogonia, mature eggs, oocytes and oogonia. They can also be produced in somatic cells. However, all cells are not equally susceptible. Cells under anesthesia are more susceptible than nonanesthetized cells, but cells in a state of undernutrition are less susceptible. Heat or cold accompanying irradiation has little or no effect. These statements are based on studies on *Drosophila*, wasps and other insects as well as on mice and on various plants, including barley, tobacco, jimson weed, maize, wheat, cotton, primroses and snapdragons. Similar effects have been found when using radium. As a practical conclusion, it is important for human beings to avoid the possibility of having mutations produced in their germ cells by irradiation. It is to be expected that, because of their recessiveness, such mutations would ordinarily fail to manifest themselves until several generations after they had been produced, causing physiologic disturbances and elusive effects that lowered the general vitality or efficiency more often than conspicuous morphologic abnormalities, which are difficult to recognize and deal with. Of course, such effects could be produced if the reproductive organs received the radiation, and if reproduction occurred subsequently to this, as when the radiation is used for purposes of temporary sterilization. These conclusions rest on the plausible premise that the hereditary material of man is affected by irradiation in a similar way to that of insects and plants.

Charles Packard, of the institute of cancer research, Columbia University, devotes a short chapter to a summary of the biologic effects of roentgen rays and radium. When roentgen and gamma radiations are absorbed in living tissues they initiate a series of changes which lead to temporary injury to the cells or to their ultimate death. The first step in the series is purely physical; the quanta of radiation collide with electrons of the atoms composing protoplasm and displace them from their normal positions in the system, or else remove them altogether. These changes lead to the breaking down of protein substances into simpler compounds which are, in a sense, foreign to the cells, with resulting alteration in the morphologic and physiologic condition of the cells. The latent period between

the moment of exposure and the appearance of a definite reaction depends on the dose and also on the kind of change which is chosen as an evidence of injury. The primary biologic effects of radiation are changes in the hydrogen ion concentration of protoplasm, in the permeability of the cell membrane, in viscosity and in the respiratory rate. The morphologic effects which accompany them are undoubtedly secondary. Cells are most sensitive to radiation during mitosis. It is a common observation that some cells which have received less than the lethal dose may recover perfectly and continue to grow and divide; the susceptibility of such cells is in inverse proportion to their recuperative powers.

The idea did not occur to the earlier investigators that radiation might promote the physiologic activities and growth of cells and tissues. There can be no doubt that some physiologic processes are quickened by roentgen and gamma irradiations, but there is no direct evidence that weak radiations are able to cause stimulation. Stephan contends that although growth is not accelerated, there are certain functional activities which are directly promoted by small doses of radiation, such as shortening of the coagulation time of the blood and increased diuresis. But in view of the fact that increased cell division following irradiation has never been proved, it seems probable that other explanations for these phenomena must be found.

Whether cells recover or die after irradiation depends not only on the number of roentgens which they receive but also on the intensity of the beam. As with any injurious agent, as soon as the cells suffer damage they begin the process of repair; if the injury is inflicted slowly, as by a beam of low intensity, the cells are more likely to recover. With high intensities of radiation, the tolerance dose is only slightly higher than the erythema dose, whereas with low intensities it is much greater.

Long experience has shown that hard rays are more selective in their action than soft rays, but this difference in action is not due to the quality of the beams so much as to their intensity. Within the last few years the weight of evidence shows that all wavelengths are equally effective. There are many who do not agree that this statement can be applied to the erythema reaction. It is a matter of common experience that the skin will tolerate a larger dose of hard rays than of soft rays.

The book is a valuable compilation of the latest views on many phases of radiology. It is to be hoped that later editions of the work will fill the gaps left by the various contributors, and supply complete annals of the science of radiology.

**Text-Book of Pathology.** By Robert Muir, M.A., M.D., Sc.D., L.L.D., F.R.S., Professor of Pathology, University of Glasgow; Pathologist to the Western Infirmary, Glasgow. Third Edition. Price, \$10. Pp. 957, with 546 illustrations. Baltimore: William Wood & Company, 1933.

The second edition of this textbook was reviewed favorably in the *ARCHIVES* (12:685 [Oct.] 1931). In the present edition the author has "endeavored to incorporate as far as possible the many and important advances made in Pathology since the previous edition was published." The number of pages has been increased from 872 to 957, and the number of illustrations from 501 to 546, but the price has been reduced from \$14 to \$10. There is no occasion for any further review at this time. The book is essentially an acceptable and useful text on the structural changes in human disease.



## Books Received

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TUBERCULOUS INFECTION IN MILK. A REPORT BY THE DEPARTMENT OF HEALTH FOR SCOTLAND. Special Report Series, No. 189. Price, 9d. Pp. 38. London: His Majesty's Stationery Office, 1933.

TEXT-BOOK OF PATHOLOGY. Robert Muir, M.A., M.D., Sc.D., LL.D., F.R.S., Professor of Pathology, University of Glasgow; Pathologist to the Western Infirmary, Glasgow. Third edition. Price, \$10. Pp. 957, with illustrations. Baltimore: William Wood & Company, 1933.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF VISION: XIII. DETERMINATION OF THE SENSITIVENESS OF THE EYE TO DIFFERENCES IN THE SATURATION OF COLOURS. L. C. Martin, F. L. Warburton and W. J. Morgan. Medical Research Council, Special Report Series 188. Price, 1s., net. Pp. 42. London: His Majesty's Stationery Office, 1933.

THE MODERN TREATMENT OF SYPHILIS. Joseph Earle Moore, M.D., Associate in Medicine, the Johns Hopkins University; Physician in Charge, Syphilis Division of the Medical Clinic, and Assistant Visiting Physician, the Johns Hopkins Hospital, Baltimore. Price, \$5. Pp. 535. Springfield, Ill.: Charles C. Thomas, Publisher, 1933.

LE POISON DES AMANITES MORTELLES. R. Dujarric de la Rivière. Price, 60 fr. Pp. 182, with 24 illustrations. Paris: Masson et Cie, 1933.

DE VENARUM OSTIOLIS, 1603, OF HIERONYMUS FABRICIUS OF AQUAPENDENTE (1533-1619). A facsimile edition of Fabricius' famous work on the valves of the veins, celebrating the quartreccentenary. With an introduction, translation, notes and reproductions of the original plates of the first edition (1633). Kenneth J. Franklin, D.M., Tutor and Lecturer in Physiology of Oriel College and University Demonstrator of Pharmacology, Oxford. Price, \$3. Pp. 104, with 15 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

L'INFECTION CHEZ LES INSECTES, IMMUNITÉ ET SYMBIOSE. A. Paillot, Docteur ès Sciences, Lauréat de l'Institut, Directeur de la Station de Zoologie agricole du Sub-Est. Pp. 535, with 275 figures. Trévoux: G. Patissier, 1933.

WILHELM CONRAD RÖNTGEN AND THE EARLY HISTORY OF THE ROENTGEN RAY. Otto Glasser, Ph.D., Director, Radiation Research Department, Cleveland Clinic. With a Chapter on Personal Reminiscences of W. C. Röntgen by Margaret Boveri (Berlin). Price, \$6. Pp. 496, with 96 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

# ARCHIVES OF PATHOLOGY

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## HISTOLOGY OF ADENOMA OF THE ISLETS OF LANGERHANS

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AND

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ST. LOUIS

In 1924, Seale Harris<sup>1</sup> presented evidence to indicate that spontaneous hyperinsulinism might result from overactivity of the islet cell tissue of the pancreas. More recently, it has been shown by several authors that comparable manifestations are due to the endocrine activity of islet cell neoplasms. Five of these tumors were removed in the surgical clinics of Washington University following preoperative diagnoses of chronic hypoglycemia; in all, the subsequent history of the patients proved the tumor to have been the actual cause of the symptoms. Detailed pathologic reports on three of the five tumors accompanied the case reports of Carr, Parker, Grove, Fisher and Larimore,<sup>2</sup> Womack, Gnagi and Graham,<sup>3</sup> and Graham and Womack.<sup>4</sup> Two of the cases are as yet unreported.

The present communication concerns the results of a comparative histologic study of these five tumors; more particularly, we have been interested in (1) genesis, (2) the appearance of the cells of each as compared with normal human islet cells, (3) the stage of development and (4) the effect of tumor secretion on the normal islets of the pancreas in which the tumor was found. The basis of such a study has been amply provided by past investigators, since accurate knowledge concerning the islets of the pancreas is not exceeded by that relating to any other endocrine gland.

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1. Harris, S.: J. A. M. A. **83**:729, 1924.

2. Carr, A. D.; Parker, R.; Grove, E.; Fisher, A. O., and Larimore, J. W.: J. A. M. A. **96**:1363, 1931.

3. Womack, N. A.; Gnagi, W. B., and Graham, E. A.: J. A. M. A. **97**:831, 1931.

4. Graham, E. A., and Womack, N. A.: Surg., Gynec. & Obst. **56**:728, 1933.

## PROCEDURE

The tumors were available for fresh study and for fixation immediately following their removal. The procedure did not vary. Following a brief inspection, the tumor was sliced in a sagittal plane and the segments were fixed in various solutions.<sup>5</sup> Bits were placed in Ringer's solution and in 1:2,000 neutral red in physiologic solution of sodium chloride for fresh examination. The position and gross characteristics of the pieces placed in the various fixatives and of the fresh specimens were noted.

In each case, portions of adjoining pancreas were adherent to the tumor. These were fixed in the same fluids and subsequently dehydrated, embedded, cut and stained with the tumor material.

For staining, we relied on hematoxylin and eosin, the Mallory azan stain, the neutral dyes of Bowie and Bensley, which color the individual granules of appropriately fixed islet cells, and aniline acid fuchsin followed by Mallory II stain. In several cases, groups of islet cells were identified following one method of staining and were drawn with the camera lucida; the slide was then destained and colored again with a different stain. This procedure served to identify and locate the characteristic coloring displayed by the specific granules of each cell with several methods; it was done for both normal islet and tumor tissue.

The bits for fresh study were utilized as rapidly as possible, usually within ten minutes following removal of the tumor. They were placed on slides in a drop of Ringer's solution and teased to particulate form, and a coverslip was applied. After a brief survey with the low power microscope, typical tumor cells were studied under the oil immersion lens and their characteristics noted. The material immersed in neutral red was similarly treated and studied.

## PANCREATIC ADENOMAS PRODUCING HYPOGLYCEMIC SYMPTOMS

The clinical histories of the five patients whose tumors are hereafter subjected to comparative study are given in case reports by Carr and his co-workers,<sup>2</sup> Womack and his co-workers,<sup>3</sup> and Graham and Womack.<sup>4</sup> For convenient reference, the essential facts are summarized in the table. Here and in the subsequent observations, the tumors (and cases) are designated by Roman numerals. In case III, two tumors were removed. The removal of *a*, a nonspecific mass, did not alleviate the hypoglycemic symptoms; at a second operation, *b*, a true adenoma, was removed with successful results.

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5. These comprised: aqueous chrome sublimate (potassium dichromate, 2.5 Gm.; mercuric chloride, 5 Gm.; distilled water, 100 cc.), modifications of the same, namely, (1) acetic chrome sublimate (5 cc. of glacial acetic acid and 95 cc. of aqueous chrome sublimate), and (2) formaldehyde chrome sublimate (10 cc. of formaldehyde and 90 cc. of aqueous chrome sublimate), Bouin's fluid (saturated aqueous solution of trinitrophenol, 75 cc.; formaldehyde, 25 cc.; glacial acetic acid, 5 cc.), Altmann's fluid (equal parts of a 5 per cent solution of potassium dichromate and a 2 per cent solution of osmic acid) and a diluted solution of formaldehyde, U. S. P. (1:10) in 90 per cent alcohol. Pieces of one large tumor were also fixed in Regaud's fluid (8 cc. of a 3 per cent solution of potassium dichromate and 2 cc. of formaldehyde) and in Bensley's acetic osmic dichromate fluid (8 cc. of 2.5 per cent solution of potassium dichromate, 2 cc. of a 2 per cent solution of osmic acid and glacial acetic acid, 1 drop).

1. *Gross Characteristics of the Tumors.*—The five tumors removed varied in size, consistency, weight and color. Tumors I and II were approximately 1 by 2 cm. in size, purple-red and friable. Tumor IIIa was ovoid, of similar color, unyielding to the touch and 1 by 0.8 cm. in size. Tumor IIIb was somewhat larger than those previously considered (2 by 1.4 by 1.2 cm.), ovoid, yellowish gray and of firm consistency. Tumor IV was small, 1.5 by 0.8 cm., and similar in color to IIIb. Tumor V was also yellowish gray and of soft consistency. The surface was nodular and in some areas felt cystic; several lesser masses were suspended from the tumor by pedicles. It was extremely large for tumors of this type (9 by 9 by 11 cm.; weight, 500 Gm.).

The internal configuration also varied. The cut surface of tumors I and II presented an appearance similar to that noted externally. Tumor

*Summary of Clinical Data Relating to the Tumors*

Tumor No.	Sex	Age	Duration of Symptoms	Severity at Time of Operation	Postoperative History
I	M	19	1 year	++	Complete absence of hypoglycemic seizures
II	M	44	2 years	++	Same as tumor I
III	M	27	3 years	A. ++++ B. ++++	Temporary relief Same as tumor I
IV	F	44	4 years	+	Same as tumor I
V	M	52	2 years	+++	Same as tumor I

IIIa had a calcified core surrounded by purple-red friable tissue. Tumor IIIb resembled a cut fibroma; embedded in the capsule was a single nodule of purple-red friable tissue, 3 mm. in diameter. Tumor IV had a necrotic central core; tumor V, a calcified core with projections toward the surface. With the exception of the calcified core, tumor V consisted of a solid mass of grayish-yellow, soft, friable tissue; no cysts were found.

The tumors differed in degree of encapsulation. Tumors I and V were well encapsulated, tumor II was not encapsulated and tumors IIIa and b and IV were partially encapsulated. Further details with regard to encapsulation are given with the description of the architecture of the tumor.

2. *Study of Fresh Cells Immersed in Ringer's Solution.*—(a) Normal Islets: The researches of Laguesse<sup>6</sup> and Bensley<sup>7</sup> showed that the appearances of the cell which serve to identify islet tissue in fixed and stained preparations are also obvious in isolated fresh cells immersed in an isotonic saline solution. Such cells, studied without fixation, nearly

6. Laguesse, E.: *Revue générale d'histologie*, Paris, A. Storck & Cie, 1908, vols. 2 and 3.

7. Bensley, R. R.: *Am. J. Anat.* 12:297, 1911.

approximate the living condition; living islet cells with blood supply intact may be studied in the pancreas of the anesthetized mouse drawn out on a microscope stage between slide and coverslip (Covell,<sup>8</sup> O'Leary<sup>9</sup>).

Bensley's<sup>7</sup> detailed description of the fresh, isolated islet cells of the pancreas of a guinea-pig serves as an adequate norm for comparison with islet cells isolated from the adenomas studied. Abstractly, comparison of the normal islet cells of one species with the tumor islet cells of another may not seem justifiable, but actually, any differences which may exist among normal cells of different species are too slight for even the most expert to detect. A summary of our own experience in the study of isolated cells of normal islets of the mouse, guinea-pig and one child of 1½ years follows:

The fresh unstained cells of normal islets are polymorphic, most frequently polyhedral or columnar. Cell boundaries are usually distinct. Embedded in the cytoplasm, and more refractile, are tiny granules of uniform size, densely packed. This contiguity of granules throughout the cytoplasm is interrupted only by occasional lipid droplets, small vacuoles and a series of granule-free canals identical with the apparatus of Golgi, which may be identified in fixed tissue after staining with special osmic acid or by the reduced silver method. The nucleus, ovoid or spherical, appears homogeneous unless intense illumination is used; then the nucleolus and lesser lumps of chromatin are apparent.

Cells of normal islets in the mouse and guinea-pig may also be subjected to *supra* or *intra vitam* staining. Supravitaly, neutral red or janus green B (dilution 1:10,000) is perfused through the aorta of an exsanguinated guinea-pig. Intravitaly, neutral red is injected intravenously or subcutaneously (from 0.1 to 0.5 cc. of a 1 per cent solution); janus green B, by dripping a 1:1,000 dilution on the exposed living pancreas.

By both *intra* and *supra vitam* methods, the end-results of staining with neutral red and janus green B are the same. The specific granules of all islet cells which come in contact with the dye are colored sharply. With neutral red, the lipid droplets of the fresh cells also stain; with janus green B, the slender filamentous mitochondria are sometimes colored, as well as the specific granules.

(b) Adenomas: In tumors I, II and V, the cells were readily isolated for fresh study; in IIIb and IV, with difficulty.

In all tumors, except IIIa, isolated islet cells were obtained which were practically identical with the previously described unstained living cells of normal islets. The haze of tiny granules studded the entire

8. Covell, W. P.: Anat. Rec. **40**:213, 1928.

9. O'Leary, J. L.: Anat. Rec. **45**:27, 1930.

cytoplasmic mass, with the exception of the areas occupied by the canals of Holmgren, occasional lipid droplets and small vacuoles. The nucleus was large and vesicular. Occasional lumps of chromatin and nucleoli were observed. In tumor V, cells also occurred in which the granules varied considerably in size and did not occupy the whole mass of cytoplasm. Cells with large nuclei, rich in chromatin, were frequent as were also binucleated cells and giant cells with several nuclei.

The abundance of material available for study in tumor V made it possible to set aside a piece of tissue immersed in cold Ringer's solution for twenty-four hours. At the end of this period, many cells had not altered appreciably from the appearance observed shortly following the removal of the tumor. This indicates that the tumor islet cells are very resistant to postmortem change. Isolated normal islet cells of the guinea-pig, in contrast, sometimes undergo marked deteriorative changes one-half hour after removal from the body (Bensley<sup>7</sup>).

It has been constantly observed in the study of tumors (first by R. R. Bensley, later by ourselves) that when neutral red is applied in 1:2,000 dilution in physiologic solution of sodium chloride, the specific granules of only a minority of the islet cells color with the dye. That the failure of the granules of the majority of the cells to color (like the cells of normal islets) is not due to mechanical difficulties is readily demonstrable (1) by the occurrence of cells with unstained granules besides other cells with colored granules, and (2) by the fact that if a piece of pancreas of a guinea-pig is immersed in neutral red simultaneously with the bit of tumor, the granules of all cells of the islets of the guinea-pig will be stained, while only those of the minority of the tumor cells are colored. The granules of the majority and of the minority of cells, therefore, differ in their affinity for neutral red, indicating a difference in the composition of the granules. In tumor V, the cells with colored granules were more numerous than in any of the other tumors studied; the granules varied somewhat in size, and in many cells those stained by neutral red and the unstained granules could be observed side by side in the cytoplasm.

The content of cells revealed by fresh study in tumor IIIa and the pocket of purple-red friable tissue in tumor IIIb will be considered separately because of the dissimilarity of the cells to those which were the principal components of tumors I, II, IIIb, IV and V. The majority of cells were macrophages, lymphocytes and fibroblasts. A minority of mast cells and polymorphonuclear leukocytes were also observed. Macrophages and lymphocytes were also seen in significant numbers during the fresh study of the unquestioned islet cells tumors.

3. *Architecture of Normal Islets as Compared with That of the Adenomas.*—(a) Normal Islets: The characteristics which serve to distinguish the islets of the pancreas from duct and acinar tissue in the

usual hematoxylin-eosin preparation are so well known that comment is unnecessary. However, the architectural relations of cells, capillaries and connective tissue framework must be understood, since it is apparent that in the formation of islet cell adenomas the tendency will be toward a repetition of these relations on a large scale.

The cells of the islet (mouse) form anastomosing cords, separated by capillary channels, which pursue a course varying from straight to very tortuous through the islets. Some capillaries traverse islet tissue in passing between one acinar lobule and another; others appear to originate from short arterioles. Frequently, venous channels extend directly from an islet to a neighboring vein. A comprehensive account of the circulatory pattern of the islets is given by Beck and Berg.<sup>10</sup>

The cell-vascular relationship is an intimate one, and two or more surfaces of a single cell may abut on the capillaries. However, the contact is indirect, since a definite endothelial membrane is usually interposed between the islet cells and the vascular channels. In occasional capillaries, this membrane is not demonstrable, but the possibility is not excluded that these may be lymphatics.

The islets are separated from the acinar tissue by a partition of connective tissue of variable thickness which appears to be more closely adherent to the surfaces of the acinar lobules than to the islet cells. In the interstitial islets, which lie free from acinar investment along the larger blood vessels, this peri-insular framework is strengthened. Reticular tendrils about the capillaries are the only projections of connective tissue into the interior of the islets.

(*b*) Adenomas: Tumor III*b*, a young, growing adenoma with excellent accommodation between the parenchymatous and the vascular elements, serves as a starting point from which to develop the architectural characteristics of the group. Study at a low magnification of a section through the center of the tumor stained with Mallory azan stain shows an interlacing stroma of collagenic tissue, a central core containing blood vessels, ducts and ducts differentiating into islet tissue and a broken capsule surrounding the tumor. The parenchyma is divided into sinuous cords and lobules of islet cells, one or two (rarely more) cell layers in thickness, separated by the stroma and by arterioles and capillaries which issue from it (fig. 1 *A*). It is to be noted that the capillaries are thin-walled, very similar in appearance to those which pervade normal islets; the only connective tissue elements usually found about them are reticular fibrils. In areas adjacent to the acinar tissue of the pancreas, the connective tissue is richer and has separated the parenchyma into islands served by the capillary vessels of penetrating arterioles. Hyalinization about, and thickening of, the walls of the smaller vessels are so seldom seen that these changes are negligible. It is apparent, therefore, that this tumor is representative of Bensley's stage I in the chronological classification of islet cell adenomas (Womack and his co-workers<sup>3</sup>).

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10. Beck, J. S. P., and Berg, B. N.: *Am. J. Path.* 7:31, 1931.

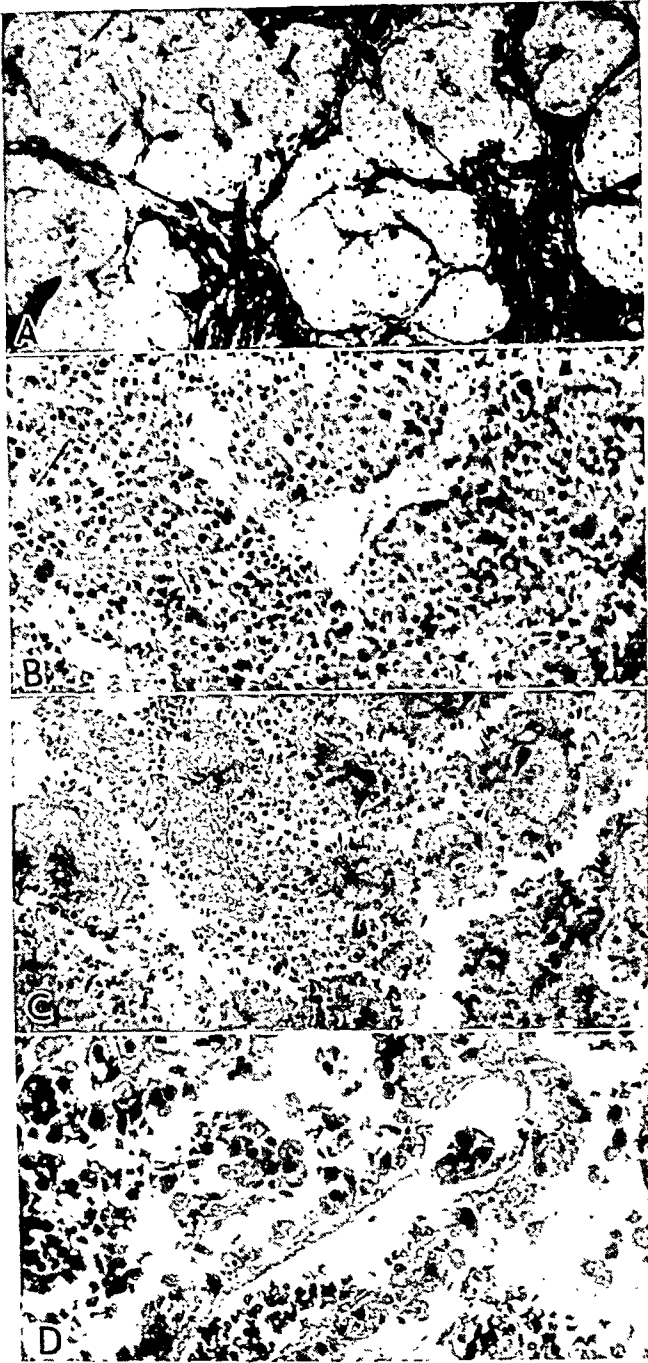


Fig. 1.—*A*, low power photomicrograph, typical area, tumor III*b*. This represents the youngest stage of tumor development observed in the series. Note the intimate relationship of cells and capillaries. *B*, from tumor V, illustrating a less perfectly organized architecture than that observed in *A*. Note the hyalinization in the vessel walls and very much less intimate cell-capillary relationship. *C*, from tumor II. The latest stage in tumor architecture observed. The vessels are severely hyalinized, and tumor cells cling to their surfaces. The intervening spaces contain blood and the debris of necrotic cells. *D*, thin-walled vascular channel from tumor V surrounded by a sheath of tumor cells. Note the looseness of the surrounding area. All photomicrographs except *D* are enlarged to 160 diameters; *D* is enlarged to 240 diameters. Zeiss 16 mm.; ocular Homal I.



Large areas of tumors IV and V are representative of a later, more disorganized stage of growth. The parenchymatous elements have increased out of all proportion to the vascular bed, resulting in the formation of thick cords and balls of cells, the center of which may be separated from the capillary vessels by three or four layers of cells (fig. 1 *B*, tumor V). Coincidentally, the majority of the small vessels have accumulated collagenic connective tissue, or their walls have thickened and the lumens become occluded by hyalinization. The latter tendency is most manifest in tumor V.

The result of this more disorganized growth has been the focal degeneration of cell groups, leaving cavities containing cellular debris and blood elements. Groups of cells with diffuse basophilic staining of the cytoplasm and pyknotic nuclei were also observed. We have been unable to find convincing evidence that these manifestations of degeneration are entirely attributable to a poor vascular supply, since they sometimes occur in the neighborhood of apparently functioning vessels. One area in tumor V suggests the end-result of this degeneration of islet tissue. Here the ghosts of former vessels, occasional functioning ones and a few masses of apparently normal islet cells occur in a dense collagenic stroma.

Although the vascular supply of the parenchymatous elements in each of tumors IV and V was not adequate as a whole, free corpuscles were sometimes observed between normal cells, and occasionally an endothelial nucleus was interposed between these and the surrounding islet cells. This may represent a belated attempt toward more efficient vascularization. Also, in small areas of both tumors IV and V, conditions reminiscent of the major area of tumor IIIb were found. Here, two and three layered cords of islet cells abutted directly on capillaries with thin walls free from hyalinization.

In tumor V, an area was found which was not duplicated in any of the other tumors studied. The only marked accumulations of connective tissue accompanied the arteries. Peculiar to it was the occurrence of numerous thin-walled vascular tubes having a single layered or multi-layered investment of parenchymatous cells (fig. 1 *D*). Sometimes edematous connective tissue intervened between the outer surfaces of the tubes and the tumor cells; mostly the cells were attached directly to the walls of the vessels. Tumor cells were infrequently located in these thin tubes and also in the larger vessels of the area. Between the cell-coated tubes there were cellular debris and recently extravasated red blood corpuscles.

Tumors I and II were characterized by the occurrence of areas of a later stage than that considered most typical of tumors IV and V. Tumor I had predominantly this arrangement; tumor II, in part. The appearance was that of anastomosing cords of hyalinized connective

tissue separated by cavities containing extravasated blood and necrotic remnants of tumor cells (fig. 1 C). Clinging to the walls of the cavities were single or multiple layers of islet tumor cells which varied in appearance; some were obviously degenerated, and many appeared to be quite healthy.

4. *Characteristics of Normal Human Islet Cells Compared with Those of Adenomas.*—(a) Normal Islet Cells: The salient characteristics of islet cells have been noted in describing their appearance in the living, unstained state. Since the fixed tissue equivalents of the cell components do not differ in material detail, we may next consider the basis of classification into cell types.

Following appropriate methods of fixation and staining, three cell types can be identified in the islets of Langerhans of the human pancreas. Two of these, alpha and beta, were first defined by Lane<sup>11</sup> in the pancreas of the guinea-pig. Lane observed that following fixation in 70 per cent alcohol, the specific granules of the alpha cells stained purple with neutral gentian; in the remaining (majority) cells of the islets the granules had dissolved and the cytoplasm remained clear. Conversely, following fixation in chrome sublimate and staining with neutral gentian, the specific granules of the second cell type (called beta by Lane) colored; those of the alpha variety did not color. Bensley<sup>7</sup> later successfully stained the specific granules of the alpha and beta cells differentially in the same islet with aniline acid fuchsin and methyl green, following fixation in his own acetic-osmic bichromate fluid. Bloom<sup>12</sup> was able in human islets, following fixation in formaldehyde chrome sublimate, to stain these two cell types and a third (D) by using the Mallory azan stain. Henceforth, the three cell types will be referred to as A, B and D.

Experimental studies (Allen<sup>13</sup> and Homans<sup>14</sup>) designed to effect exhaustion of the cell type of the islets, active in the production of insulin, through overwork of a greatly reduced pancreas resulted in hydropic degeneration of the B cells; the A variety remained normal. Restoration of the normal appearance of the exhausted B cells of such overworked islets has also been brought about by the administration of proper doses of insulin (Copp and Barclay<sup>15</sup>). Control pieces of pancreas, taken before the institution of insulin therapy, showed the B cells to be in an advanced state of hydropic degeneration. These data, together with inferences concerning the relative alcohol solubility of the granules of the A and B cells, indicate that the B variety is active in the production of insulin.

The three cell types occur constantly in human islets. Bloom finds that the B cells are much more numerous; A and D cells occur in about

11. Lane, M. A.: *Am. J. Anat.* 7:409, 1907.

12. Bloom, W.: *Anat. Rec.* 49:363, 1931.

13. Allen, F. M.: *J. Metab. Research* 1:1, 1922.

14. Homans, J.: *J. M. Research* 30:49, 1914.

15. Copp, E. F. F., and Barclay, A. J.: *J. Metab. Research* 4:445, 1923.

equal numbers. In our material the average frequency of occurrence is in the order B, A and D.

Material fixed in formaldehyde chrome sublimate was used for all of our comparisons between the staining reactions of the specific granules of normal and tumor islet cells. After staining with Mallory azan stain, normal islet A cell granules colored deep red; B cell granules, lilac to gray-yellow; D cell granules, light blue. Bowie's<sup>16</sup> neutral stain colors the granules of the normal islet B cells deep blue;<sup>17</sup> those of A and D cells, light blue, pink, or not at all. Normal islet B granules color purple; A and D granules do not color with Bensley's neutral gentian. However, when a section is stained by Bowie's or Bensley's neutral dyes and destained and Mallory azan stain is applied, it is apparent that some overlap occurs. Some of the cells designated as A cells in preparations stained with Mallory azan stain may appear to be B cells after being stained with Bowie's dye. This discrepancy suggests that those comparisons which necessitate assigning types to islet cells be made by the Lane<sup>11</sup> method.

Differential staining of the specific granules by the Lane method is the one good criterion for assigning types to islet cells. Other cell characteristics, which have been said to differ from one type to another, are the shape and chromatin content of the nucleus, sharpness of cell outline, granule density and position of the cells relative to the capillaries. However, in nonspecifically stained preparations (man) these characteristics are hardly sufficient to distinguish one type from another.

Islet cells give a negative reaction for enterochromaffin (argentaffin) granules with Masson's method.<sup>18</sup> This is of importance since the argentaffin cells of the gastro-intestinal epithelium may also form tumors. The type enterochromaffin cell is cylindric or flask-shaped; the basal portion of the cytoplasm (between the nucleus and the capillary pole) is studded with granules in size similar to the specific granules of the islet cells. In some animals, though not usually in man, these are more uniformly distributed throughout the cytoplasm. The granules reduce silver by the specific method employed by Masson for material fixed in Bouin's fluid; the specific granules of the islet cells do not. Van Campenhaut,<sup>19</sup> using a block method of silver impregnation, recently succeeded in demonstrating that the specific granules of certain of the islet cells, as well as enterochromaffin granules, reduce silver. None of our tumors or normal pancreases were so prepared.

(b) Tumor Islet Cells: Just as the parenchymatovascular accommodation in tumor IIIb appeared the most perfect in our series, so the great majority of tumor cells more closely approximated the appearance

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16. Bowie, D. J.: *Anat. Rec.* **29**:57, 1924.

17. Bensley: Personal communication to the authors.

18. Masson, P.: *Am. J. Path.* **4**:181, 1928.

19. Van Campenhaut, E.: *Proc. Soc. Exper. Biol. & Med.* **30**:617, 1933.

of those of normal islets than in any of the other adenomas studied. The healthy appearing cells, of which this tumor had a large majority, presented a slender columnar, flask-like or polyhedral appearance, depending on their position in the sinuous cords and lobules (fig. 2 A). The nuclei were similar in size and chromatin content to those of normal islet cells. Most often the cytoplasm appeared to be evenly studded with specific granules, with the exception of the areas occupied by the canals of Holmgren and cytoplasmic inclusions (p. 294); sometimes they were concentrated at the capillary poles. In tumors I and II, the healthiest appearing cells were very similar to those just described. In tumors IV and V, polyhedral or rounded cells predominated. The nuclei varied in size from normal to one-half again as large, and also in chromatin content. The specific granules were evenly distributed in many cells; in others they were concentrated about the nucleus, leaving a granule-free zone at the periphery. No significant difference in size of the granules of a single cell was apparent. Binucleated cells and giant cells with central clusters of nuclei were not uncommon.

Reference to the appearance of tumor islet cells, observed fresh after *supra-vitam* staining with neutral red, makes it apparent that at least two types of cells occurred in the tumors: The majority had granules which did not color with neutral red; the granules of the minority did. Sometimes, particularly in tumor V, colored and uncolored granules were observed in the cytoplasm of a single cell. Differences in the colors assumed by the specific granules of a majority and minority of cells were detected in fixed and stained preparations. It is well to state immediately that we do not presume the types of cells which can be demonstrated supravivally by neutral red to be identical with those apparent in fixed and stained preparations. It is true that in each procedure the numerical ratio of one type to the other may be similar (tumor IIIb) and also that each division is based on the staining characteristics of the specific cytoplasmic granules. But for obvious reasons, it is not possible to apply the supravital and fixed tissue methods to the study of a single group of cells. To avoid confusion, therefore, we speak hereafter of the majority cells of fixed and stained preparations as type I cells, the minority cells as type II.

In a preparation from tumor IIIb fixed in the formaldehyde chrome sublimate and stained with Bowie's dye, the distinction between type I and II cells was most evident. The specific cytoplasmic granules of type I cells colored faint blue; those of the type II cells stained an intense blue. There was a similar difference between the two types when Bensley's neutral gentian was used. Following the use of Mallory azan stain, the specific cytoplasmic granules of type I cells colored lilac to pink; those of the type II cells, purple. A third variety of cell was

observed in preparations fixed in the formaldehyde chrome sublimate and stained with Mallory azan stain. The specific cytoplasmic granules colored red. Owing to the infrequent occurrence of these cells in tumor IIIb, and their nonoccurrence in the other tumors, they deserve but passing mention.



Fig. 2.—*A*, characteristic cell group from tumor IIIb. Note the intimate relationship of cells to the capillary channels, cytoplasmic inclusions (*cyt.*) and intracellular canals (*i.c.*). Formaldehyde chrome sublimate; Mallory azan. *B*, acinar cells becoming differentiated from duct epithelium in area of regenerating pancreatic tissue at the edge of tumor IIIb adjoining the pancreas. Acinar cells are indicated by *a.c.*; duct elements, by *d.c.* *C*, intimate relationship of duct and islet tissue in the vicinity of the central core, tumor IIIb. Duct cells are indicated by *d.c.* All photomicrographs are enlarged to 1,220 diameters.

In tumor IIIb, type II cells were fairly numerous and occurred singly or in clusters of from two to eight. In tumors I and II, they

were found in similar proportions. In tumor IV, type I cells were infrequent, and their specific granules somewhat larger than in tumors I, II and IIIb. In tumor V, the type II cells were not observed.

Can the type I and II cells of the adenomas be said to correspond to the major cell types of normal islets? Study of the material at our disposal does not warrant the conclusion that they can. We prefer to accept R. R. Bensley's decision that the type I cell is a B cell with modified staining reactions; the type II cell, a B cell corresponding to those of normal islets.

In preparations stained with hematoxylin and eosin the granules of the parenchymatous cells of the enterochromaffin and islet cell tumors are both eosinophilic and of approximately the same size. Besides, the cell form and appearance of the nucleus may present striking similarities. This led us to carry out the Masson test for enterochromaffin granules on tumors II, IIIb, IV and V. In each case, a piece of intestine known to contain enterochromaffin cells was fixed in Bouin's fluid and carried through the procedure with the tumor slide. In every instance the Masson reaction was positive for the granules of the enterochromaffin cells of the intestine and negative for the specific granules of islet cells.

Cytoplasmic inclusions of variable shape, but giving similar staining reactions, have been found in each of the tumors studied (fig. 2 A). Their occurrence in islet cell adenomas was detected by R. R. Bensley (Womack and his co-workers<sup>3</sup>). The inclusions were of most frequent occurrence in tumor IIIb, less often found in tumors I, II and IV and rare in tumor V. With Mallory azan stain, they colored bright red; with Bowie's stain, blue and with Bensley's stain, purple. They might be plastered on one surface of the nucleus ("sickle thickening," Bensley) or appear as globules or masses in the cytoplasm. In tumor IIIb, observed in mitotically active cells, they colored the same as the chromatin. These have been found only in tumor cells of type I, but never in type II, normal islet or acinar cells.

Mitotic figures were most frequently observed in type I cells of tumor IIIb. They usually occurred in cells with a reduced content of specific granules. In tumor V, only occasional mitotic figures were observed; in tumors I, II and IV, none.

In normal islets, cellular degeneration is of infrequent occurrence. Occasional cells with pyknotic nuclei and basophilic cytoplasm are observed, but there is no obvious equilibrium between cellular degeneration and replacement. There was little evidence of degeneration in tumor IIIb. Cells with pyknotic nuclei were found in small numbers, and occasional cell clumps were noted in which the nuclei were filled with droplets of nucleolar size. In tumors I, II, IV and V, cellular degeneration was frequent as evidenced by pyknosis, diffuse basophilia

of the cytoplasm and (tumor V) clumping of the mitochondria into droplets. Tumors I and II each had areas in which cell groups had been converted into débris. The cytoplasmic inclusions described have not been found in degenerating cells.

In all of the five tumors described, the majority of cells were sufficiently well differentiated to possess the characteristics of mature cells. In V, tumor cells in blood vessels, variability in the size of the cells and the many bizarre nuclear forms rich in chromatin were indicative of malignancy. However, this was probably of low grade, and by many the tumor undoubtedly would be classified as benign.

5. *Duct Components of Islet Cell Adenomas.*—Ducts occurred in all of the adenomas, which were similar in cell characteristics and arrangement to the small and medium-sized pancreatic ducts. They were least frequently met with in tumor V, but its large size did not permit a complete survey. The ducts occurred in the central cores of tumors IIIb and IV, in the connective tissue trabeculae of these and in tumors I and II.

Inferential evidence of the differentiation of duct into islet tissue was found in tumors I to IV (fig. 2 C). Notable areas were those about the central cores (IIIb and IV) and immediately adjoining normal pancreas. In these, hollow cords of islet cells frequently contained duct elements; single islet cells were found interspersed among the cells of ducts, and granules similar in size and staining characteristics to those of islet cells sometimes spotted the cytoplasm of an obvious duct cell. Similar sporadic occurrences were noted in the vicinity of the connective tissue trabeculae.

In the areas adjoining the pancreas in tumors IIIb and IV, evidence of the differentiation of duct into acinar tissue was also obtained (fig. 2 B). Sometimes ducts terminated in incomplete acini; again, cells containing a few zymogen granules and basophilic material (prozymogen?) were noted in the walls of ducts. Simple lobules, possibly in the process of building, were also seen. Differentiation of duct into acinar tissue did not occur elsewhere in the tumors.

6. *Islets of the Pancreas in Cases of Hypoglycemia.*—It might be expected that an overproduction of the hypoglycemic hormone would alter the appearance of the pancreatic islets. In all of the cases of tumor, portions of the pancreas adjacent to the tumors were available for comparison with the normal by the same variety of fixing and staining methods used in the study of the tumors. This survey included comparison with the normal, the following criteria being used: the proportion of alpha, beta and delta cells, richness of cells in specific cytoplasmic granules and evidence of degeneration. Apparent differences in the number of islets was not considered a good criterion (Bensley<sup>7</sup>). No

alterations could be demonstrated without the range of expected variability. A segment of pancreas removed from a 1 year old child (Dr. E. Graham) for chronic hypoglycemia also had normal appearing islets.

#### LITERATURE

Islet cell adenomas are not of rare occurrence. In 1926, Warren<sup>20</sup> collected fifteen cases from the literature and reported four others. All were discovered at necropsy. Since the hypoglycemic syndrome was not widely understood before that time, it is not surprising that the early tumors reported were not correlated with positive or negative evidence of hypoglycemia in the patients' histories.

Seale Harris<sup>1</sup> first suggested that the blood sugar level might be lowered and hypoglycemic symptoms become apparent owing to abnormal activity of the islet tissue of the pancreas. Since that time islet cell tumors have been discovered at the necropsy of patients who had had hypoglycemic symptoms. These cases have been reported by Thalhimier and Murphy,<sup>21</sup> McClenahan and Norris,<sup>22</sup> Smith and Seibel,<sup>23</sup> Terbrüggen,<sup>24</sup> Barnard,<sup>25</sup> Rabinovitch and Barden,<sup>26</sup> Cairns and Tanner,<sup>27</sup> and Wolf, Hare and Riggs.<sup>28</sup>

In two of the five cases reported by Smith and Seibel,<sup>23</sup> there was no history suggestive of hypoglycemia; in fact, one of the patients, during an attack of acute lobar pneumonia which shortly preceded death, "behaved as a total diabetic."<sup>29</sup> The parenchyma of the tumor had an arrangement similar to that of islet tissue; but the appearance of the cells suggested a closer relation to duct than to islet tissue. The specific cytoplasmic granules characteristic of islet cells could not be demonstrated although the fixation was adequate (acetic and formaldehyde chrome sublimates). The other patient died of a primary liver cell carcinoma. In many cells of the tumor specific cytoplasmic granules, which stained as do the B granules of normal islets, were demonstrable.

The complete case reported by Wilder, Allen, Power and Robertson<sup>30</sup> first suggested operative intervention in spontaneous hypoglycemia

20. Warren, Shields: *Am. J. Path.* **2**:336, 1926.

21. Thalhimier, W., and Murphy, F. D.: *J. A. M. A.* **91**:89, 1928.

22. McClenahan, W. V., and Norris, G. W.: *Am. J. M. Sc.* **177**:93, 1929.

23. Smith, M. G., and Seibel, M. G.: *Am. J. Path.* **7**:723, 1931.

24. Terbrüggen, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **88**:37, 1931.

25. Barnard, W. G.: *J. Path. & Bact.* **35**:929, 1932.

26. Rabinovitch, J., and Barden, F. W.: *Am. J. M. Sc.* **184**:494, 1932.

27. Cairns, R. M., and Tanner, S. E.: *Brit. M. J.* **1**:8, 1932.

28. Wolf, A.; Hare, C. C., and Riggs, H. W.: *Bull. Neurol. Inst. New York* **3**:232, 1933.

29. Death resulted from a pulmonary embolus which originated in the femoral vein.

30. Wilder, R. M.; Allen, F. N.; Power, M. H., and Robertson, H. E.: *J. A. M. A.* **89**:348, 1927.



due to suspected islet cell tumor. The first successful operation was reported by Howland, Campbell, Maltby and Robinson.<sup>31</sup> Since that time there have been five operative removals of adenomas of the islets of Langerhans at Barnes Hospital, St. Louis. Dr. A. O. Fisher removed two such tumors (one reported by Carr, Parker, Grove, Fisher and Larimore;<sup>2</sup> one diagnosed by Dr. L. F. Aitken, not yet reported); Dr. Evarts Graham, three (Womack, Gnagi and Graham;<sup>3</sup> Graham and Womack,<sup>4</sup> one not yet reported). Dr. E. R. Schmidt at the University of Wisconsin has removed one (Bast, Schmidt and Sevringhaus<sup>32</sup>). In each of these cases there was a successful outcome with complete alleviation of symptoms.

Architecturally and from the point of view of cellular differentiation, the pancreatic tumors giving rise to hypoglycemic symptoms have all resembled the islets of Langerhans. However, it may not be inferred that all tumors with well differentiated islet cells provoke hypoglycemic symptoms. One of the two cases without symptoms reported by Smith and Seibel illustrates this point. Doubtless other such tumors will be discovered at necropsy. The factors which may condition the severity of symptoms are enumerated on page 305.

#### COMMENT

The genesis of the islet cell adenoma is of particular interest in view of the researches of Bensley<sup>33</sup> and Grauer<sup>34</sup> on the origin of islet and acinar epithelium from the pancreatic ducts in young adult animals. Bensley has shown in rabbits that following ligation of the duct of Wirsung the acini and islets degenerate. Coincident with and following this degeneration, there is a restoration of islet tissue through the building of new islets from the ducts. Grauer demonstrated the regeneration of acinar epithelium from the ducts by similarly ligating the main duct, waiting until the acinar epithelium was completely destroyed, as proved by histologic control, and then reestablishing the duct connection with the intestine. With the free drainage of the duct system, the phenomenon of regeneration of acini from the epithelium of the duct was observed. In several cases the pancreas of young adult rabbits was completely regenerated. The epithelium of the duct is thus totipotent, capable of forming either acinar or islet tissue.

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31. Howland, G.; Campbell, W. R.; Maltby, E. J., and Robinson, W. L.: *J. A. M. A.* **93**:674, 1929.

32. Bast, T. H.; Schmidt, E. R., and Sevringhaus, E. L.: *Acta chir. Scandinav.* **71**:82, 1932.

33. Bensley, R. R.: *Harvey Lectures*, Philadelphia, J. B. Lippincott Company, 1915, vol. 10, p. 250.

34. Grauer, T. P.: *Am. J. Anat.* **38**:233, 1926.

In the central cores of tumors IIIb and IV of our series, ducts were plentiful. Centrally located ducts were also observed in the tumor reported by Howland and his co-workers.<sup>31</sup> Despite the occurrence of ducts in the trabeculae of tumor IIIb and crossing into it from the normal acinar tissue adjoining, no evidence was obtained to indicate that the central mass of ducts was connected with the ducts of the pancreas, though it is to be presumed that all ducts found in the islet tumors are of this origin. This question can be settled only by the study of serial sections through a small adenoma.

Around the central cores of tumors IIIb and IV, in the projections of the ducts into the parenchyma and also in the central cores themselves, inferential evidence of the differentiation of ducts into islet tissue was found (p. 304). It seems likely, therefore, that the ducts of the central core in these tumors served as one focal point for the differentiation of islet tumor tissue and may indeed have been the ultimate origin of a major portion of it. That differentiation from duct tissue alone does not explain the increase in size of the tumors is proved by the numerous mitoses observed in the islet cells of tumor IIIb.

At the outskirts of tumors II, IIIb and IV, in contiguity with normal pancreas, ducts were also present. In tumor IIIb, the capsule was broken in this area by the passage of ducts from normal pancreas into the tumor. In all those mentioned, differentiation of duct into both islet tumor and apparently normal acinar tissue was observed. However, evidence that a single duct might differentiate into both acinar and islet tissue could not be obtained. This, and the failure to find forming acini elsewhere in the tumors, might be taken to indicate that the epithelium of the duct, while totipotent, may be restricted in its ability to form either islet or acinar tissue by environmental or other (intracellular) influences.

Evidence concerning the likeness of tumor cells to A and B cells of normal islets must be examined with care. This question cannot be settled until the results from a much larger series are weighed. A survey of the present material suggests definitely that the type I cells of the adenomas have tumor characteristics; but in order that the staining characteristics of the tumor as compared with normal islet cells can be finally evaluated, future material must be fixed in 70 per cent alcohol and Hermann's fluid in addition to the fixatives previously prescribed. Bowie's and Bensley's stains should be applied to material fixed in these fluids and in aqueous chrome sublimate and the results compared with those of Lane.<sup>11</sup>

Our uncertainty regarding which cells of the tumors have granules that color with neutral red has already been alluded to. In tumor V, the study of fixed material would lead one to believe that all cells belonged to the same category (type I). Yet, in the fresh study, neutral red

stained the specific granules of a larger number of cells than in any of the other tumors, and cells were met not infrequently which had both colored and uncolored granules.

Tumor IIIb had numerous type II cells, yet fewer fresh cells proportionately stained with neutral red than in tumor V. It is at least evident, as previously intimated, that the type II and I cells of fixed material do not correspond, respectively, to fresh cells which do and do not stain with neutral red.

The stages of hyalinization as observed in the islets of the pancreas ("the most typical pancreatic lesion in diabetes mellitus," Warren<sup>35</sup>) and in the islet cell adenomas offer striking parallels. In both, the hyalin appears first about the vessels and pushes the cells progressively farther away from the vascular channels. Two possible origins of the hyalin of the islets have been discussed. Those favoring an epithelial origin point to the occurrence in the islets of cells the cytoplasm of which colors blue with Mallory stain. In a later stage, intracellular hyaline droplets are said to appear, these fusing to form hyaline masses about the capillaries. Warren pointed out, however, that the cells with blue-stained cytoplasm after Mallory's connective tissue stain appear in normal islets; and Bloom,<sup>12</sup> using the Mallory azan method, has found them so constantly that he has designated them as a third cell type, the D cell. Warren further stated that "the serial section method shows that the masses of hyaline are always in contact with the walls of the vessels of the islands, and always intercellular." He concluded: "Hyalinization of the islands of Langerhans . . . may be considered as due to production of intercellular substance by fibroblasts and possibly by endothelial cells." The evidence afforded by study of hyalinization in islet cell adenomas supports Warren's conclusions. Furthermore, the D cells with blue-staining cytoplasmic granules have not been found in the adenomas of our series.

Certain inferences may be drawn between the histologic appearance of the islet cell adenomas of our series and the amount of hypoglycemic hormone each was capable of producing, as judged by the severity of preoperative symptoms and (in two cases) by the insulin assay of a portion of the tumor. The patient having tumor IIIb showed the most severe hypoglycemic symptoms of the series. While the tumor was not large, it was actively growing; the majority of cells deviated to the least degree from the beta cells of normal islets; the cells immediately adjoined capillary channels. Next in order of severity of symptoms was the patient having the 500 Gm. tumor V. The ratio of weight between IIIb and V was in the order of 1:250. The appearance of the majority of

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35. Warren, Shields: *The Pathology of Diabetes Mellitus*, Philadelphia, Lea & Febiger, 1930.

cells deviated more markedly from that of the beta cells of normal islets than in any other tumor of the series; the cells were most frequently separated from the vascular channels by accumulations of hyalin. The insulin assay was relatively low. The patients having tumors I and II grade next in order of severity of symptoms. These tumors, like IIIb were small and had areas in which the cells were widely separated from the vascular channels by hyaline masses; areas of degeneration were apparent in each. However, the majority of healthy appearing cells deviated little from the beta type; and tumor II had an area in which the healthy appearing cells closely adjoined the capillaries as in IIIb. The patient having tumor IV had the least severe symptoms of hypoglycemia. The tumor was the smallest of the group; it contained a considerable area of degeneration, and a majority of the cells deviated markedly in appearance from normal beta cells, though not as much so as in tumor V. Summarizing, then, the apparent factors which determine the severity of symptoms are: (1) degree of similitude of the majority of cells to the beta cells of normal islets; (2) the access of cells to the vascular channels; (3) size of the tumor, and (4) evidence of degeneration. Another factor which must be considered is the functioning of the normal islets of the pancreas.

The evidence developed by Bensley and confirmed by us indicates that these adenomas may have an age cycle. Do some of them degenerate into nonspecific masses after a period of active existence? Tumor IIIa, a nonspecific mass with a calcified core, removed from the pancreas that lodged IIIb, was very similar in histologic appearance to a nodule of reddish friable tissue which occurred in the capsule of tumor IIIb (p. 298). The possibility is evident that the nonspecific mass, IIIa, may have been an active tumor which produced hypoglycemic symptoms in the patient before the development of the apparently young tumor IIIb (Graham and Womack<sup>4</sup>). Removal of this mass gave little temporary relief, and after three days did not result in even a partial amelioration of symptoms.

Changes must be sought for in the pancreatic islets of patients having islet cell adenomas producing hypoglycemic symptoms. Such alterations would be useful if they proved specific. A biopsy on the pancreas in a suspected case without visible or palpable evidence of a tumor at the exploratory operation might then prove an excellent diagnostic procedure. Any alterations found must be analyzed with reference to the patient's carbohydrate tolerance after operative removal of the adenoma, since adenomas have been found in the pancreases of diabetic patients (Büchner<sup>36</sup>).

36. Büchner, F.: *Klin. Wchnschr.* **11**:1494, 1932.

## SUMMARY

Five tumors of the pancreas, operatively removed, have been identified as islet cell adenomas, verifying in each case the preoperative diagnosis of hypoglycemia due to suspected tumor. Although the adenomas varied in size, gross appearance and apparent age, the majority cell type of each was closely allied to the beta cells of the normal islets of Langerhans, but possessed definite tumor characteristics. In only one of the five tumors was there evidence of malignancy.

The staining reactions of the specific cytoplasmic granules in the majority of tumor cells of each deviated sufficiently from those of the beta cells of normal human islets to lend support to the hypothesis of dysinsulinism.

In none of the cases did the histologic picture of the islets of Langerhans of the pancreas containing the tumor indicate the suppression of function that might be expected to parallel the prolonged secretion of excessive amounts of the hypoglycemic hormone.

# MALFORMATIONS OF THE HEART OF THE NEW-BORN

CONGENITAL LESIONS SUGGESTIVE OF AN INFLAMMATORY ORIGIN

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Etiologically, congenital lesions of the heart can be explained either on a developmental or on an inflammatory basis. Formerly most abnormalities were considered the result of inflammatory processes, and lesions, cicatrized or active, in the region of the malformation were assumed to be of inflammatory origin. It was not until Rokitansky, introducing the term "fetal endocarditis," laid down certain rigid microscopic criteria that the rarity of lesions with an inflammatory genesis was appreciated. They occur more frequently in the left side of the heart. In 1907, Mönckeberg<sup>1</sup> could collect from the literature but 12 reports of such cases, to which he added a case of his own. Since then, Fischer,<sup>2</sup> Nagel,<sup>3</sup> Loeser,<sup>4</sup> Kockel,<sup>5</sup> Ganef<sup>6</sup> and Le Tulle<sup>7</sup> have described similar cases on an inflammatory basis with marked stenosis of the aortic opening.

Keith, on the contrary, on the basis of a study of 270 cases of congenital defects of the heart, reached the conclusion, because of lack of scars and because of the concomitant anomalies in other parts of the body, that changes are the result, not of infection, but of a disturbance of the conditions under which the fetus develops. Vierordt<sup>8</sup> estimated that other abnormalities, such as harelip, syndactylism, horseshoe kidney and hypospadias, are found in about 10 per cent of the cases of congenital heart disease.

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From the Clinic of the Woman's Hospital.

1. Mönckeberg, J. G.: *Verhandl. d. deutsch. path. Gesellsch.* **11**:224, 1908.
2. Fischer, B.: *Frankfurt. Ztschr. f. Path.* **7**:83, 1911.
3. Nagel, W.: *Ein Beitrag zur Kasuistik ueber angeborene Herzfehler*, Dissert., Freiburg, 1908.
4. Loeser, A.: *Virchows Arch. f. path. Anat.* **219**:309, 1915.
5. Kockel, R.: *Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte, Koeln* **80**:39, 1908.
6. Ganef, M.: *Ueber angeborene Stenose und Atresie der Aorta durch foetale Endokarditis*, Dissert., Würzburg, 1910.
7. Le Tulle: *Presse méd.* **22**:432, 1914.
8. Vierordt: *Angeborene Herzkrankheiten*, in Nothnagel: *Specielle Pathologie und Therapie*, Vienna, A. Hölder, 1898.

Rauchfuss<sup>9</sup> emphasized that septal defects constitute the main indication of a developmental anomaly. The absence of septal defects, however, does not always predicate an inflammatory origin, as is shown by descriptions of cases of cor pseudotriloculare without septal defects, in which the developmental origin cannot be doubted.

Septal defects occur much less commonly in association with aortic stenosis and atresia than with the corresponding lesions of the pulmonary valve; according to Vierordt, there are about three times as many cases of aortic stenosis and atresia without, as with, septal defects. Theremin<sup>10</sup> collected 17 such cases, in only 2 of which there was a defect of the septum, and in Abbott's<sup>11</sup> series of 6 cases there was no septal defect in 5. It is obvious that in cases without septal defect, the determination of whether the genesis was developmental or inflammatory is much more difficult. In the cases in which the septum is open, it must be recalled that the aortic valve is completely formed in the seventh week of intra-uterine life, and the intraventricular septum by the end of the eighth week; hence, in order to explain the nonclosure of the septum, it must be assumed that the process of stenosis, if due to inflammation, commenced just at the end of the seventh week, and ceased before the end of the eighth week, when the septum normally closes. This, as Vaquez<sup>12</sup> stated, is inadmissible.

Another factor which is contradictory to the theory of an inflammatory origin is that congenital lesions of the heart may be hereditary and familial. De la Camp found almost identical cardiac malformations in 2 sisters and 4 brothers. Burwinkel described the case of a man of 54, who had been cyanotic since birth, as were his mother and grandmother and 2 of his 3 children.

According to Rauchfuss,<sup>9</sup> the time of development of a lesion must be placed before the twentieth week of life if a recessus formation of the right ventricle (a funnel-shaped extension of this cavity, pointing to the left and surrounding the left ventricle) is encountered.

In general, the size of the right ventricle in relation to that of the left is an indicator of the time of occurrence of the lesion; the earlier a disturbance takes place, the larger will be the right ventricle, which, with a few exceptions, hypertrophies in response to functional demand and carries the burden of the whole circulation.

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9. Rauchfuss, C.: *Die angeborenen Entwicklungsfehler des Herzens und die Foetalkrankheiten des Herzens und der grossen Gefaesse*, in Gerhardt: *Handbuch der Kinderkrankheiten*, Tübingen, H. Laupp, 1878, vol. 4, p. 1.

10. Theremin, E.: *Etude sur les affections congénitales du coeur*, Paris, Asselin & Houseau, 1895.

11. Abbott, M. E.: *Congenital Heart Diseases*, in Osler, William, and McCrae, Thomas: *Modern Medicine*, ed. 3, Philadelphia, Lea & Febiger, 1927, vol. 4.

12. Vaquez, H.: *Diseases of the Heart*, Philadelphia, W. B. Saunders Company, 1924.

Inflammation is possible as soon as vessels appear in a system, and although vascularization occurs early in the embryo, it is Fischer's<sup>12</sup> opinion that the rare occurrence of inflammation in early intra-uterine life is due not to failure of development of the vessels but to a special immunity of the embryonal system against infection. In the early period the organism lacks cell receptors for toxins; as intra-uterine life progresses, the receptors develop, and as gestation reaches its end, the embryo is much more susceptible to infections. Ribbert<sup>13</sup> emphasized the remarkable fact that in congenital syphilis, in which there is often an enormous growth of spirochetes in the fetal body, infection of the valves of the heart does not take place. Syphilitic changes in the valves have never been found in the new-born (B. Fischer and Herzheimer). In general, therefore, it appears that the fetal valves are seldom the site of infectious lesions. In the early years of extra-uterine life also this specialized immunity seems to hold good. In 1,000 autopsies on children under 3 years of age, Ribbert found not a single case of endocarditis. Other observers, however, have reported a few cases occurring in this age group. In the second decade of life, as is well known, the number of endocardial infections increases enormously.

The present view thus tends to assign the majority, if not all, of the congenital defects primarily to abnormalities of development. However, in the following report of 2 cases of morphologically similar abnormal hearts in new-born infants, the results of detailed histologic examination tend to prove the inflammatory nature of the lesions.

#### REPORT OF CASES

**CASE 1.—History.**—A boy was born, by low forceps delivery, of a healthy primipara, 28 years old. The prenatal period was uncomplicated. The baby lived forty-eight hours, during which he showed severe progressive cyanosis and respiratory embarrassment.

**Gross Postmortem Examination.**—There were no external abnormalities. The internal organs were in normal position, and there were no abnormalities except in the heart, which occupied the largest part of the thoracic cavity. The pericardium was glistening and contained about 10 cc. of clear fluid. The anterior aspect of the heart and the apex were formed by the right ventricle; the left ventricle was small and appeared as an appendix to the right. The incisura cordis was more than 1 cm. deep and was situated to the left and posteriorly. Both auricles appeared large. The longitudinal sulcus was situated near the left margin and was only 1.5 cm. long. The posterior aspect of the heart was supplied mainly by the right ventricle, which measured at its widest 3.3 by 3.2 cm., while the left measured only 2.2 by 1 cm.

The large vessels originated normally. The pulmonary artery, which originated without a distinct conus, was 2.5 cm. wide when opened. The branches to both lungs were given off in the normal way. The ductus Botalli was 1.3 cm. wide. The

13. Ribbert, H., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol. 2.



descending aorta appeared as the continuation of the pulmonary artery. Below the origin of the right carotid artery the aorta was 1.5 cm. in diameter; the aortic ostium, however, was narrow, being less than 0.5 cm. in diameter. An attempt to enter the aorta from the ventricle failed, as the probe was arrested by an elastic resistance at the level of the aortic valve. This obstruction was due to the coalescent leaflets of the aortic valve which formed a folded membrane on the inferior surface of which were the fused noduli arantii. A narrow slit, 2 cm. long, in the center of the membrane was the only communication between the aorta and the left ventricle. In addition, the lateral portion of the membrane, at its upper margin, was intimately adherent to the aortic wall. The sinuses of

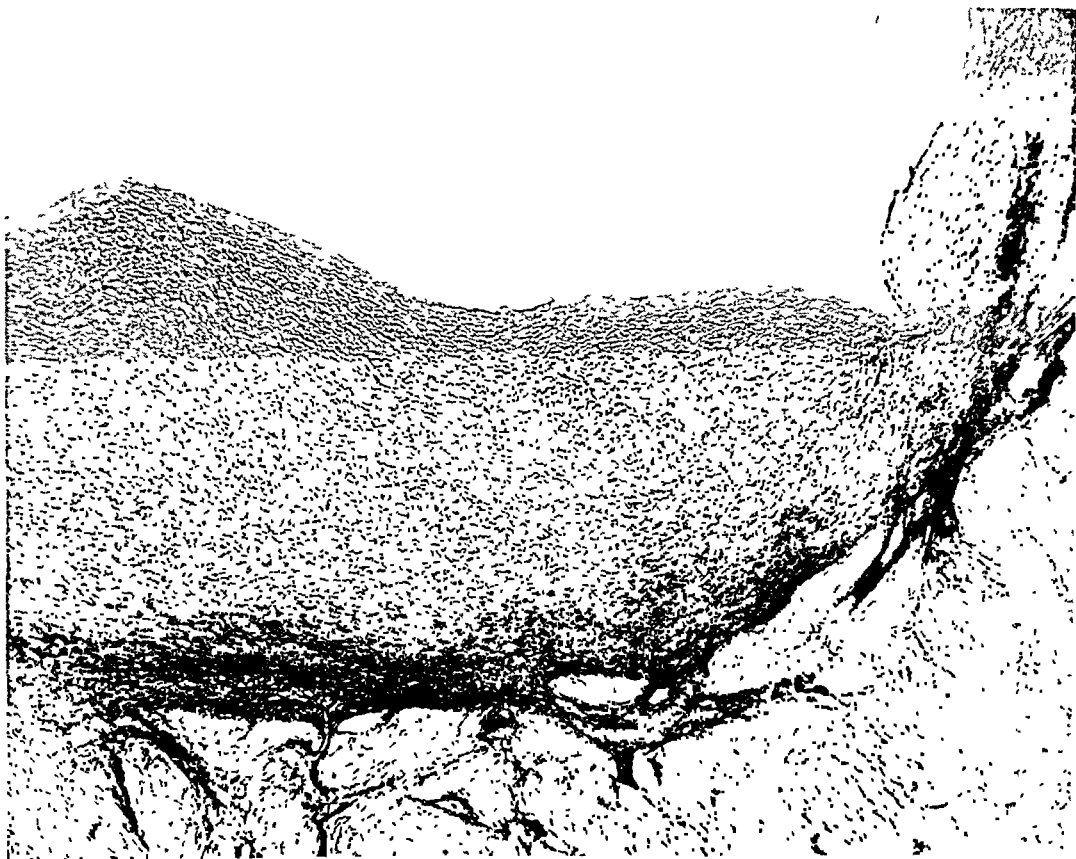


Fig. 1 (case 1).—Thickened endocardium of the left ventricle (elastica stain).

Valsalva appeared high and flat. The commissures were unusually long. The coronary arteries originated normally; their orifices were rather wide.

The right ventricle appeared normal from inside; the medial papillary muscle, however, was only 6 mm. long, while the others measured from 1.2 to 1 cm. The thickness of the wall, without trabeculae, was 3 mm.

The left ventricle as a whole was turned backward, almost forming a recessus to the right ventricle. The endocardium here was transformed into a pale grayish, smooth layer of an average thickness of 1.5 mm. The anterior papillary muscle was partly coalescent with the endocardial thickening, only a short upper portion extending from the wall. The mitral valve was represented by the posterior cusp only, as the aortic cusp was lost within the thickening of the wall, causing shortening of the corresponding papillary muscle. The chordae tendineae

were short anteriorly and long posteriorly, and the spaces between them were partly filled by a reddish, gelatinous tissue. The intima of the left auricle was normal. The foramen ovale was patent, and its diameter was about 5 mm.

The epicardium did not show anything of note.

*Histologic Postmortem Examination.*—Left Ventricle: The thickened endocardial layer (fig. 1) consisted of elastic connective tissue and in places attained a thickness equal to a third of the myocardium. The borderline between the endocardium and the myocardium generally was well marked; in many places, however, the elastic layer projected into the muscle with long extensions that continued along the course of the blood vessels. The elastic fibers were arranged

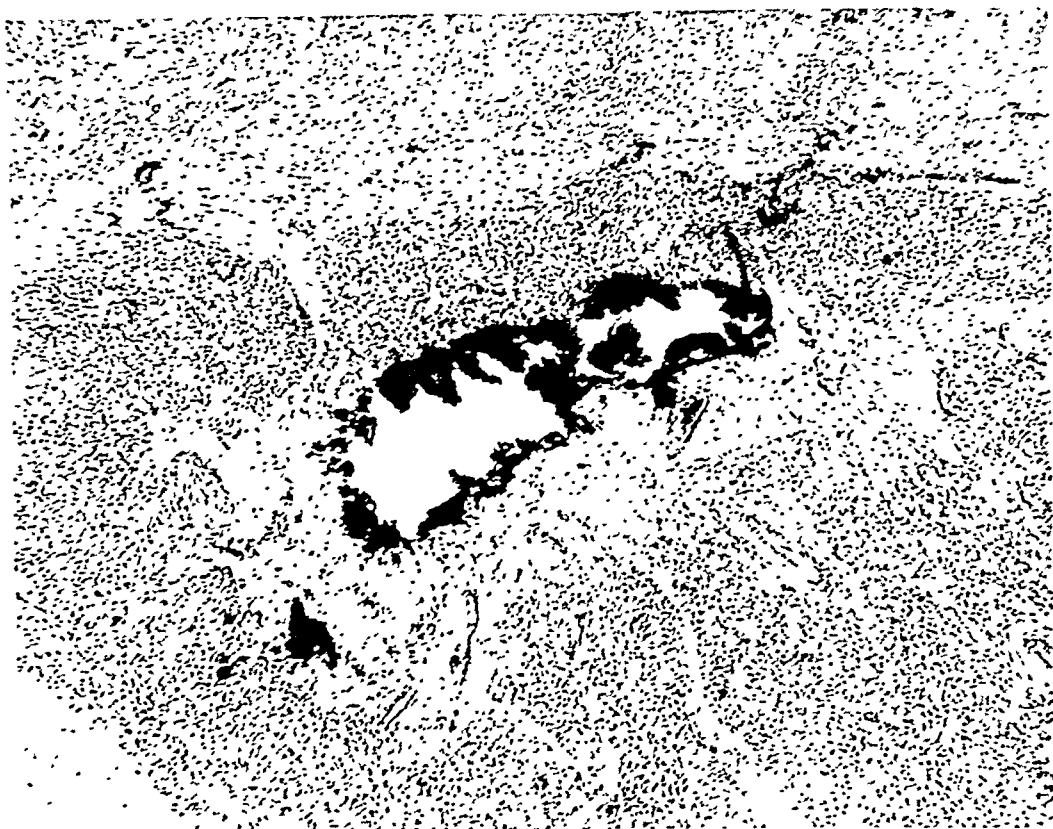


Fig. 2 (case 1).—Calcification in the myocardium of the left ventricle.

parallel to the inner myocardial surface and were coarser and denser in the basal layer in the parts projecting into the myocardium. Congested capillaries were found in the endocardial thickening close to the myocardium. Near the papillary muscle there was a large necrotic area with extensive central calcification. Multiple calcified deposits were found in interstitial spaces occupied by hyalinized connective tissue (fig. 2). In the adjacent muscle tissue the capillaries were increased in number and engorged.

The fibers of the heart muscle showed in places irregular arrangement, atrophy and vacuolation. The nuclei varied considerably in size and shape, a condition which might be attributed to the embryonic stage of the muscle. Aggregations of small round cells were frequently seen (fig. 3), and numerous small necrotic areas and islands of cicatricial connective tissue were found in the muscle sub-

stance; there were considerable thickening and hyalinization of the blood vessel sheaths and extravasation of blood.

Right Ventricle: There were numerous localized necroses and some round cell infiltration in all layers of the heart muscle; there was no increase of the elastic tissue. The muscle cells varied in size and structure and were somewhat irregularly distributed. The sheaths of the walls of the blood vessels were thickened in many instances.

Left Auricle: Endocardial thickening of the same type as in the left ventricle, though of lesser extent, was found, likewise with projections into the myocardium. The muscle exhibited marked irregularity in structure.



Fig. 3 (case 1).—Small round cell infiltration in the myocardium of the left ventricle.

Right Auricle: The endocardium was normal; the myocardium showed irregularities of the muscle tissue.

Septum: There were no changes aside from structural irregularity of the muscle.

Papillary Muscle of the Left Ventricle: Necrosis occupied most of the center, with extensive calcification, as described in connection with the left ventricle. The adjacent muscle tissue was preserved only in fragments, and there were marked increase and engorgement of the capillaries. A broad peripheral layer composed of elastic connective tissue formed the outer area. The extent and shape of the calcification did not suggest an origin in a former blood vessel.

**Mitral Valve:** In cross-section (fig. 4) the valve represented an irregularly folded band and consisted of loose, regularly arranged connective tissue with scanty elastic fibers. The endothelium was well preserved in areas. The opposite wall of the ventricle showed the endocardial and muscular changes that have been described. There was no trace of an active or chronic inflammatory lesion.

**Aortic Valve:** The different parts of the valve showed variation in density without inflammatory changes. Elastic tissue could not be demonstrated within the valve proper, since the elastic surface layer of the wall of the ventricle ends at the origin of the valve.

The right atrioventricular valve, the pulmonary valve and the aortic wall did not show unusual appearances.



Fig. 4 (case 1).—Transverse section through the mitral valve near the free margin, showing fusing and clumping of cusps.

**CASE 2.—History.**—A boy, born at full term, by spontaneous delivery, of a healthy secundipara, 28 years old, lived for nine days, during which time he presented the clinical symptoms of cyanosis of various intensity with attacks of apnea.

**Gross Postmortem Examination.**—The patient was a new-born boy, 54 cm. long, with all the external signs of maturity. There were no abnormalities except cyanosis and scattered petechial hemorrhages.

When the chest was opened the lungs were found to be pushed backward by the heart, which was much enlarged and occupied the left side and part of the right side of the thoracic cavity. The pericardial sac contained about 5 cc. of clear, serous fluid and showed a good sheen.

The heart presented an unusual appearance. The anterior aspect was almost entirely made up of the right ventricle and auricle, the latter being greatly distended and congested. The left ventricle was about a third of the size of the right and simulated a dome-shaped protrusion of the right ventricle. The incisura cordis was shifted to the left and was about 0.5 cm. deep. The apex consisted entirely of the right ventricle. The main measurements of the heart were: width at the base, 5 cm.; width of the right ventricle at the base, 3.7 cm.; longest distance from the top of the right auricle to the apex, 5.2 cm.; distance from the base of the left ventricle to the apex, 4.2 cm. The longitudinal sulcus was shallow and entirely effaced near the base; it measured 2 cm. in length. There was a

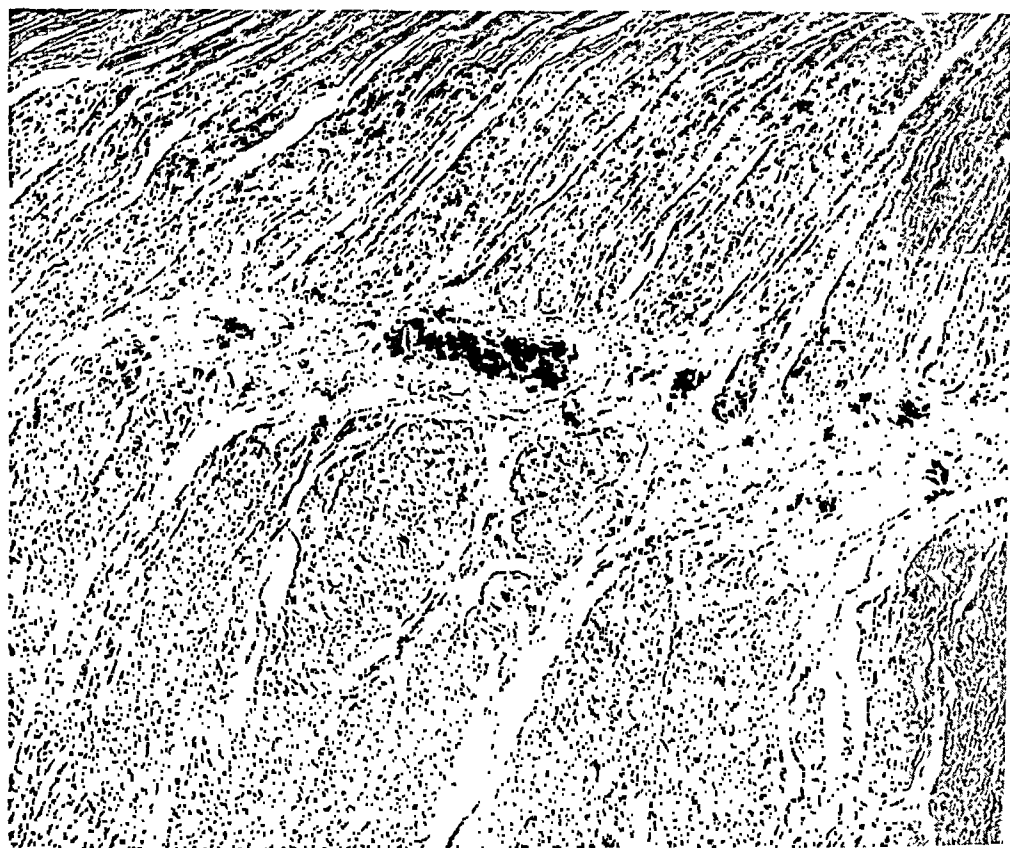


Fig. 5 (case 2).—Diffuse calcifications in the myocardium of the left ventricle.

subserous hemorrhage about 1 cm. in diameter at the base, near the origin of the longitudinal sulcus. Numerous pinpoint-sized subserous hemorrhages were scattered over the pulmonary conus and the ascending aorta. The configuration of the large vessels was unusual. The pulmonary artery in its ascending portion formed an aneurysmal dilatation, with a widest diameter of 9 mm., which after a course of 10 mm. gave off its two branches. The main stem, however, continued with a narrowed diameter of 5 mm. upward to the aorta, thus corresponding to a large ductus Botalli. It united with the aorta after the latter had given off the left subclavian artery. The aorta originated normally. In its ascending portion it formed a wide arch, which varied in width in its different parts but, on the whole, was considerably narrower than the pulmonary artery. It measured 6.5 mm. in diameter at its origin, 4 mm. between the origin of the innominate artery and that of the left carotid artery and dilated to a diameter of 5.5 mm. before

its junction with the wide portion of the pulmonary artery, referred to as the ductus Botalli. Owing to the inversion of the normal proportions in the dimensions of the two vessels, it seemed as if the aorta would empty into the pulmonary artery. From here on the aorta widened suddenly, attaining a diameter of 8 mm., and formed a second arch in its descending portion. The arch of the aorta and the pulmonary artery could easily be probed from the descending aorta through the ductus Botalli. The main vessels to the head and the arms originated in the normal way.

Examination of the chambers and valves revealed the following: The wall of the left ventricle was uniformly 9 mm. thick; the external layers were homogeneous, and the inner layers were of coarser texture, with intertwining, whitish fibers. The inner surface of the whole ventricle was invested with a dense, whitish layer, varying in thickness from 1 to 2 mm. The inner architecture of the ventricle was largely effaced; the papillary muscles were obliterated; the trabeculae carneae could be vaguely distinguished under the endocardial coat. The mitral valve presented only one free leaflet (the aortic); the short chordae tendineae fused directly with the endocardium; the second leaflet was completely merged with the endocardium and its contours could be distinguished only vaguely. The atrioventricular ostium was moderately stenosed. The left auricle was small and of normal shape, and the endocardium did not reveal an unusual appearance.

The wall of the right ventricle was 6 mm. thick, enlarging to double this width in the area of the conus. The cavity was greatly enlarged, forming a bulging pouch in its anterior upper portion, which corresponded to the distended conus. The tricuspid valve was normally developed. The right auricle did not present any unusual appearance except for moderate dilatation. The endocardial layer in both right chambers appeared normal. The aortic ostium was narrowed, and the aortic valve was composed of two triangular flaps attached at their bases to the ostium and projecting with free edges upward into the aortic lumen; the flaps were composed of gelatinous tissue with smooth surfaces, thrown into longitudinal folds. The lumen of the ostium was almost occluded by these structures, which left an opening only 1.5 mm. in diameter. The pulmonary ostium appeared normal.

The other organs did not reveal unusual changes.

*Microscopic Postmortem Examination.*—Left Ventricle: As in case 1, the endocardium was converted into a broad layer of noncellular elastic connective tissue, which in some areas was more than half the thickness of the muscle wall. The borderline toward the myocardium was irregular and frayed; the muscle receded, and the connective tissue projected into the myocardium with finger-like extensions frequently following the course of large blood vessels. This unusual endocardial thickening was continued in the left auricle, but ended gradually at some distance from the origin of the mitral valve. Large blood vessels in dense distribution, occupied or surrounded by calcified depositis, were found near the apex in the endocardial coat where it reached its largest extent. In an area nearby extensive necrosis with calcification was encountered in the muscle; in its environs vascularization and congestion were most extensive.

The subendocardial muscle layer was composed of atrophic fibers with wide interstitial spaces. There were marked irregularity of the muscle cells, particularly at the site of the origin of the mitral valve, and general hyperemia and engorgement of the capillaries, which were greatly increased in number in this part of the myocardium. In an area of the septum the subendocardial muscle layer was intersected by a strand of hyalinized connective tissue with numerous

scattered calcified deposits of varying extent, some of them apparently surrounded by remains of vessel walls (fig. 5). Round cell infiltration, necrosis or connective tissue scars were not found in this part of the myocardium. The muscle fibers became more regular in appearance and arrangement toward the periphery of the ventricular wall. The sheaths of the blood vessels in all parts showed extensive thickening and hyalinization. The most extensive pathologic changes in the myocardium were evident near the attachment of the papillary muscle of the left ventricle where subendocardially diffuse necroses and irregularities of muscle fibers and nuclei were apparent. The tissue in the distal portion of the muscle gradually became regular, and the attachment of the mitral valve was normal.



Fig. 6 (case 2).—Transverse section through the tricuspid valve near the free margin, showing calcification.

The mitral valve in longitudinal section presented an irregularly folded band composed of loose connective tissue with irregular cellular distribution. Some elastic fibers were found at the edges, and in some other areas there were clumps of opaque matter apparently corresponding to the gelatinous material seen grossly on the surface of the valve. The endocardium was well preserved in many places. There was no trace of inflammation as evidenced by round cell infiltration or vascularization, and there were no necroses or areas of condensed stroma indicative of a previous lesion.

The tricuspid valve was built of very loose connective tissue; the ground plate was little developed. On the upper edge of the atrial surface there was a large, knoblike body composed of loose connective tissue in the center and a

circular border of dense calcified deposits suggestive of derivation from a productive inflammatory process (fig. 6).

The aortic valve showed uneven contours, and several finger-shaped projections extended from it toward the aortic lumen. The ground plate was thicker than usual. The endocardium of the aortic surface was thickened and fused imperceptibly with the central plate of connective tissue; it contained large blood vessels which in a longitudinal section of the valve appeared as a chain of transverse sections. The endocardium of the ventricular surface did not show vascularization.

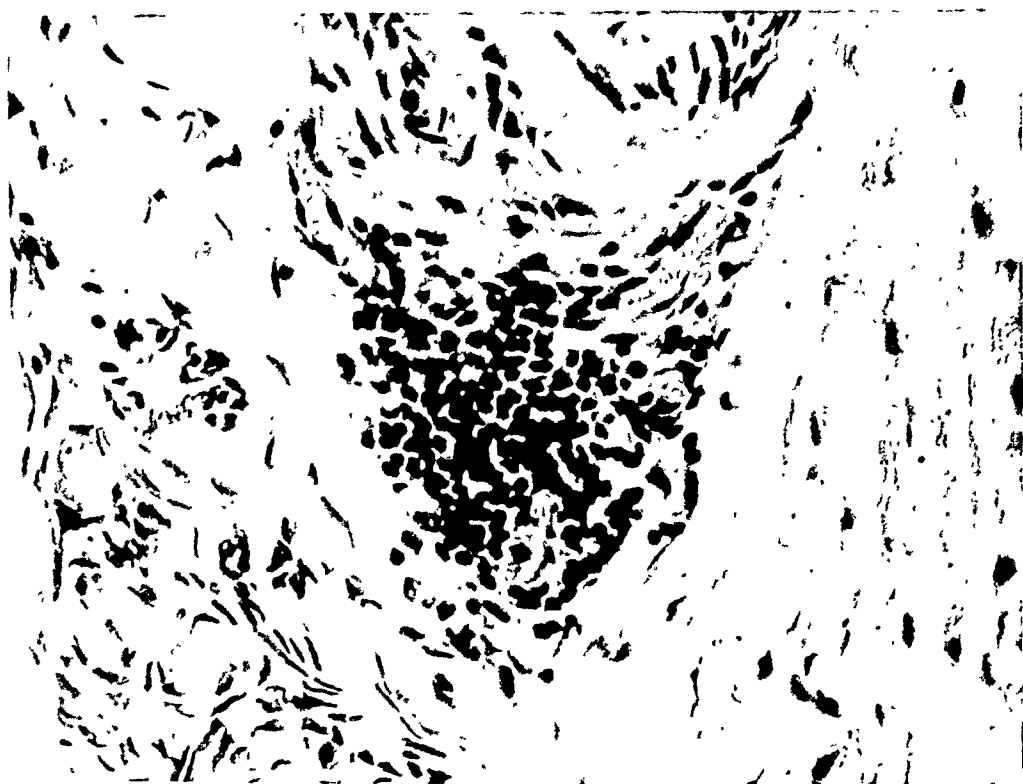


Fig. 7 (case 2).—Aggregations of round cells in the aortic adventitia.

The aorta and the pulmonary artery did not reveal structural alterations; however, dense aggregations of round cells were encountered in two places in the adventitial tissues of the aorta (fig. 7).

The lungs showed slight congestion and partial atelectasis. There was mild fatty degeneration of the liver. The kidneys showed congestion.

#### COMMENT

In evaluating the etiologic factors, macroscopic and microscopic changes are taken into consideration, but the histologic picture only is accepted as the final criterion for diagnosis.

In both cases the abnormalities were confined to the pathologic changes of the heart. This condition points to an etiologic factor acting



locally, since in the presence of developmental abnormalities in one organ multiple abnormalities in other organs commonly occur. A hereditary etiologic factor cannot be traced in either case since the parents and families of both infants were free from congenital abnormalities; the mother of the patient in the first case subsequently gave birth to two healthy babies. This further corroborates the theory of an inflammatory genesis, because in a high percentage of developmental lesions of the heart a familial predisposition can be demonstrated.

In both cases the malformation was evident chiefly in the left side of the heart; there was involvement of the proximal portion of the aorta, but the interventricular septum was completely developed, without defects. In the combined lesion of malformation and endocarditis which is represented by these two cases, involvement chiefly of the left ventricle corresponds, according to Rauchfuss, to the postnatal type of primary endocarditis, since in cases of developmental deformities of the heart with secondary endocarditis the inflammatory lesion is invariably seated in the right side of the heart.

The absence of a septal defect in both cases indicates that the development of the abnormalities must be assigned to a period later than the seventh or eighth week of intra-uterine life because the septum normally is closed by this time. Furthermore, at this early stage embryonal tissue is not considered to be susceptible to infection.

These morphologic changes, which are generally considered indicative of an inflammatory genesis, were abundantly substantiated by the histologic observations. In both cases there was an almost identical thickening of the endocardium, projecting into the myocardium, with extensive formation of elastic connective tissue. This picture corresponds to the phase of healing in a postnatal inflammatory condition and consequently indicates a reparative process in these cases. The extensive development of new capillaries and capillary leukocytosis are usual concomitants or sequelae of inflammation. In both cases the most extensive lesions were found within the papillary muscles (similar to Fischer's case), where necroses and calcifications indicate the past influence of toxic processes. That the papillary muscle is the seat of the most prominent lesion is apparently the result of increased functional demand. Round cell infiltration and capillary leukocytosis were found within the myocardium only in case 1; they may be considered as proof of an inflammatory genesis. The presence of focal necrosis and connective tissue scars furnishes additional support for this assumption.

That traces of past inflammation were least distinct in the valves proper can be explained by the fact that the valvular connective tissue possesses great regenerative power, particularly during fetal life. Consequently, in our cases the connective tissue of the valves was only

slightly inflamed or not diseased, while the endocardium on their surfaces showed extensive pathologic changes and the valves proper revealed severe deformities. Regarding the time of occurrence of the lesion, there was no apparent illness of the mother or any fault of environment which would give a clue to the transmission of infection. The onset of the disease obviously can be placed in an earlier period in case 1 than in case 2, since in the former instance the morphologic changes were much more extensive and there was obviously a greater disproportion between the right and the left ventricle, a condition which is based on a longer period of functional compensation and led in this case to a recessus formation of the left ventricle.

#### SUMMARY

Two cases of malformation of the heart and aortic stenosis in the new-born are described. In view of the anatomic and histologic observations the malformations may be considered secondary to a primary fetal inflammation.

# COLLOID-CHEMICAL PROPERTIES OF SOME PROTEIN-AMINE COMPOUNDS

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When the water-holding capacity of living tissues<sup>1</sup> was first declared to be dependent on their content of hydrophilic colloids (chiefly proteins), it followed as a corollary that edema<sup>2</sup> is merely a state in which the normal degree of hydration is heightened. A cause of edema, then, was any agency which under the conditions operative in the living organism might so affect the (protein) tissue colloids that they acquired an increased capacity for swelling. First in this category was placed an abnormal accumulation or production of acids in the involved tissues,<sup>3</sup> and second, the accumulation of certain nitrogen compounds,<sup>4</sup> particular emphasis being laid on the amines, especially those which, biologically speaking, are poisonous.

This paper deals further with the second class of compounds. Their effects on a pure protein (casein) received this detailed study because of the importance which such an action is believed to have for the better understanding of what happens in the "inflammatory" types of edema. The acid factor alone yields a fairly complete explanation of the swelling that characterizes the noninflammatory edemas (such as those of heart disease, carbon monoxide poisoning and intoxication with various metals); and the therapeutic reduction of such edemas through the use of alkalis and of various neutral salts may be taken as proof of the correctness of the general concept. But in the inflammatory types (such as those encountered in conjunction with local or generalized infections) mere neutralization of acid with alkalis and salting leave a considerable fraction of the edema unaffected. The primary cause for this fraction of the edema must, therefore, be different, as must also

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1. Fischer, M. H.: *Physiology of Alimentation*, New York, John Wiley & Sons, Inc., 1907, pp. 187 and 267; *Am. J. Physiol.* **20**:330, 1907; *Arch. f. d. ges. Physiol.* **124**:69, 1908; **125**:99, 1908.

2. Fischer, M. H.: *Physiology of Alimentation*, New York, John Wiley & Sons, Inc., 1907, p. 268; *J. A. M. A.* **51**:830, 1908.

3. Fischer, M. H.: *Edema*, New York, John Wiley & Sons, Inc., 1910, p. 99; footnote 2.

4. Fischer, M. H.: *Science* **38**:486, 1913; *Kolloid-Ztschr.* **14**:215, 1914.

be the type of the increased hydration observed. We have looked on the action of the various amines that are produced in infected areas (and produced also as the degradation products of sterilely injured protoplasm) as such a second cause for swelling and have seen the explanation of the therapeutic failure of alkalis and salts on this fraction of the edematous process in their inability to dehydrate the amine hydrations of protein. The next paragraphs present in summary further facts bearing on the general problem, discussing, in particular, the effects of various amines on the swelling, the liquefaction and the solution of a water-insoluble protein, casein.

#### EXPERIMENTS

In these experiments we employed a casein of stock origin, free from other proteins, carrying about 10 per cent of water and practically ashless.<sup>5</sup> After low temperature drying, it absorbed (in our terminology "dissolved" or "combined with") 2.12 times its own weight when thrown into water. The control tubes in the following experiments illustrate this initial swelling of the native, or neutral, protein. Its solubility *in* water (better expressed by saying its "miscibility" with water as judged by the production of an optically homogeneous mixture) was so low that the liquid above the swollen casein mass gave no reaction to the ordinary tests for protein. For purposes of further discussion it is necessary to keep these concepts of the solubility of the protein *for* water and that of its solubility *in* water carefully apart. The two properties are totally different,<sup>6</sup> and while in certain lyophilic colloid systems (particularly those of the liquid-liquid type as opposed to those of the solid-liquid type) the passage from one element to the other may be perfectly smooth (as when blood serum or any of the liquid soaps is mixed with water), solution of the protein in water is not a mere continuation of swelling. This fact will appear again in the experiments to be described.

For purposes of orientation the effects of adding increasing amounts of an acid to a standard weight of neutral casein in the presence of a given quantity of water are shown in *A* in the accompanying illustration. In this experiment, as in all subsequent ones, the mixtures were made in the cold, the photographs showing the appearance of the reaction mixtures at the end of twenty-four hours. Except for the control tube (0) which contained 12.5 Gm. (an aliquot portion) of protein with 100 cc. of water, the remaining tubes carried equal volumes of increasingly stronger solutions of hydrochloric acid (from hundredth-normal to tenth-normal). Progressively greater swelling may be noted in tubes 3 and 4; in the remaining tubes the mixtures set to solid jellies which,

5. Harris' casein with 0.59 per cent ash.

6. Fischer, M. H.: *Science* 42:223, 1915.

with an increase in the acid, became progressively more (optically) homogeneous. As commonly said, the casein in these tubes had "gone into solution," but that this was only partially true is proved by the fact that the resulting contents of the tubes were still as viscid as egg white.

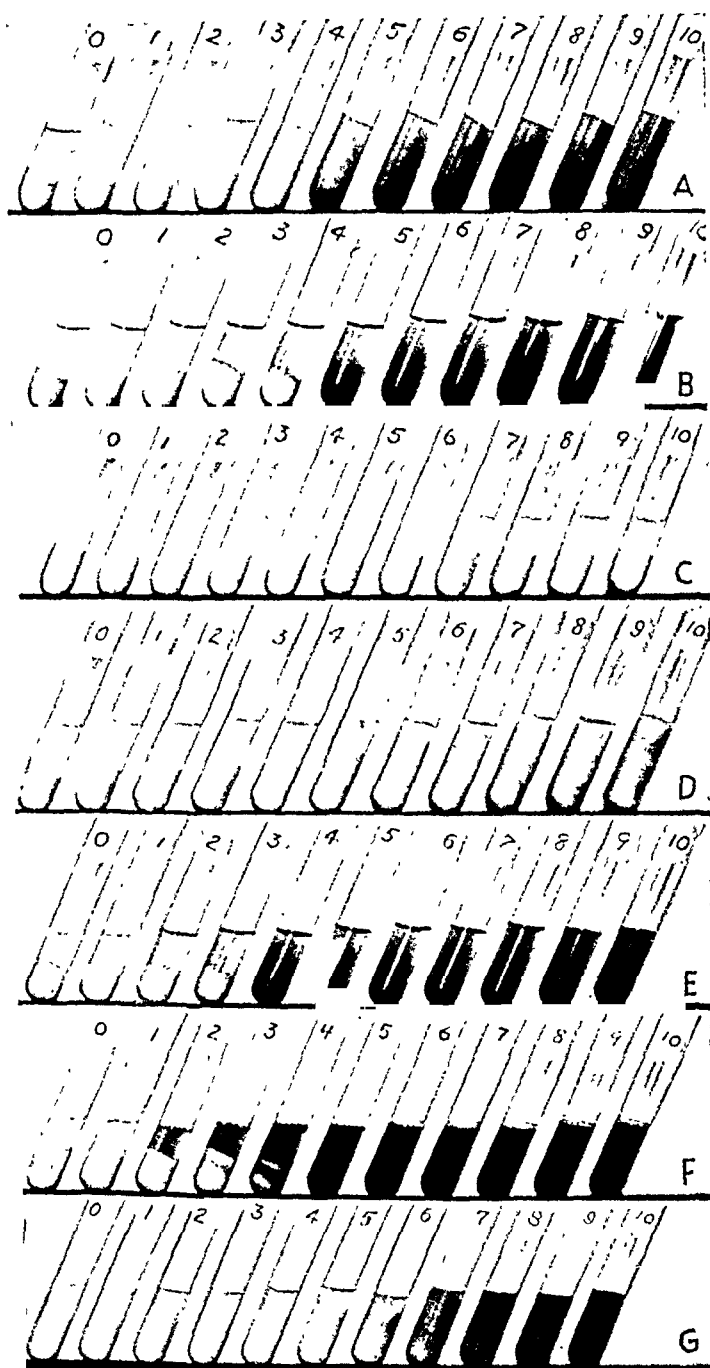
The experiment shown in *B* in the illustration brings out the same effects for an alkali, ammonium hydroxide. On comparison with *A*, it is noted that a region of swelling can hardly be discovered, the casein at once passing into solution from tube 4 on.

The effects of adding equal volumes (5 cc.) of increasingly stronger solutions of an alkyl amine, methylamine (again in this experiment and in all others referred to hereinafter, in decimal increments from hundredth-molar to tenth-molar), to a given weight of casein (0.625 Gm.) were identical with the effects of ammonium hydroxide, shown in *B*. An increase in swelling was again hardly noticeable, but the degree of solution increased steadily until in the final tube an apparently homogeneous mixture (readily miscible with any amount of added water) was obtained. This was still a liquid and hydrated colloid, however, for in spite of its optical homogeneity it was as viscid as thick glue.

An identical statement may be made regarding the primary ethyl, propyl and butyl amines as well as for the secondary methyl, ethyl and propyl amines and the tertiary methyl and ethyl amines. It may also be said of the quaternary tetramethylammonium hydroxide. As the series is ascended (either as to the complexity of the alkyl group or as to the number of hydrogen atoms replaced) the amines become increasingly insoluble in water. In the instance of such compounds as dibutylamine or tributylamine, therefore, the solubility in water will not allow of the preparation of such concentrations as are represented in the tubes shown in *B*. Nevertheless, when the proper amounts of these amines were mixed with the protein and water the ultimate result (attained in three days instead of one) was the same. We attribute this to the fact that with sufficient time even water-insoluble amines combine with the protein (as do the water-soluble amines) and the resulting compounds then swell and dissolve as do the lower members of the series. The biologic significance of this fact will be emphasized.

The amine grouping produces its effect regardless of whether it exists as free ammonia or as the primary, secondary or tertiary substitution product thereof. But when these compounds are united with acid their effects on protein disappear. Thus, when ammonium chloride is used instead of ammonium hydroxide the protein is left unaffected; and the same is true of the amines listed, the hydrochlorides of which are all without action, as shown in *C* of the illustration (methylamine hydrochloride).

When the passage is made from the alkyl amines just listed to the aryl amines, namely, phenylamine (aniline), tolylamine (toluidine), xylylamine (xylidine), naphthylamine and ethylphenylamine, both the



The colloid-chemical changes induced in unit weights of casein in the direction of its increased swelling, softening, optical clarity and solution. The successive series show the effects of standard concentrations and amounts of hydrochloric acid (*A*), ammonium hydroxide (*B*), the salt of an amine, methylamine hydrochloride (*C*), aspartic acid (*D*), paraphenylenediamine (*E*), benzylamine (*F*), and pyridine (*G*).

swelling and the solution effects on the casein, while still present, are greatly reduced. When the compounds are converted into the hydrochlorides even this slight action disappears.

Change of the alkyl or aryl amines to their corresponding hydroxyl-alkyl or hydroxylaryl amines leaves their original activity unimpaired.<sup>7</sup> But if the oxidation of the modifying hydrocarbon group is carried further, as to the aldehyde stage (instanced by the carbonyl amine commonly called formamide) or through this to the carboxyl stage (thus yielding the type picture of an amino-acid), all swelling and solvent properties disappear. From a chemical point of view, this neutral action is explained, perhaps, by the fact that a free amine group is balanced by a carboxyl group. The monocarboxyl alkyl amines, such as monocarboxylmethylamine (glycocoll), monocarboxylethylamine (alanine), monocarboxylpropylamine, monocarboxylisobutylamine (leucine), monocarboxylheptylamine and 1-carboxyl 2-hydroxylphenylethylamine (tyrosine), are without effect on casein. Several of the members of this class are the amino-acids split from protein when normally digested. Reasoning biologically, one would not expect that such compounds, constituting as they do a portion of man's daily food, would manifest any toxic properties as judged by the effects of amines on the hydration or liquefaction of a native protein; and they do not. Some support for this point of view may be found in the action of aspartic acid. This produces marked swelling of casein, as seen in *D* of the illustration. Chemically, this 1-2-dicarboxylethylamine has its one amine group offset by two carboxyl groups (and the more definitely acid nature of the compound comes into play). While aspartic acid is frequently listed as one of the normal digestion products of protein, it seems probable that the real form in which it is broken out of protein is as its amide, namely, asparagine. And asparagine is without effect on casein.<sup>8</sup>

The inactivity of the monocarboxyl alkyl amines is maintained even when the  $\text{NH}_2$  group is made to replace an OH in the carboxyl group instead of an H on one of the carbon atoms of the mother acid (as in the normal amino-acids that may be split out of protein). We thus found the amides of formic, acetic, propionic, lactic, valeric, stearic, aspartic and benzoic acids to be without action. Urea, which in higher concentration produces both swelling and liquefaction of casein, appears at first sight an exception to this rule, for it is ordinarily classed as the

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7. When the oxygen is inserted between the alkyl and amino groups, however, these hydrating effects disappear. Alphamethylhydroxylamine, for example, is without action, whereas its isomer, hydroxylmethylamine, is strongly active.

8. When only one of the carboxyl groups of aspartic acid is neutralized with ammonia, its swelling effect disappears (because the acid nature of the compound is reduced), to reappear when both groups are neutralized (for the balance of the compound is now on the amine side). Glutamic acid, which is 1-3-dicarboxylpropylamine, acts like aspartic acid.

diamide of carbonic acid. However, it may perhaps be looked on more correctly as aminoformamide, and its hydrating and solvent action may be dependent on the maintenance of the basic amine properties in the compound as thus conceived.

What amounts to the activation of an otherwise inert compound is illustrated in the effects of decarboxylation on histidine. Histidine, which is without effect on casein, becomes strongly active as soon as it is changed to histamine. This chemical degradation has its analog in the decarboxylation of any of the alpha amino-acids, such as the changing of glycine to methylamine or of alanine to ethylamine.

Change from a mono-amine to a diamine increases the hydration and solution effects of the compound on protein. Thus hydrazine and methylenediamine are more active than the corresponding mono-amines (ammonia and methylamine). This action appears even in the aryl amines. While the phenylmono-amines are without marked action, the phenylenediamines are very active. But in these the position of the amino groups is of great importance. While orthophenylenediamine produces some swelling and solution of casein this effect is small in comparison with that of metaphenylenediamine. The tremendous action of paraphenylenediamine is indicated in *E* of the illustration, an observation which explains, through direct action on protoplasm and without recourse to vascular change, the long known and almost specific pharmacologic action of this compound in the production of fatal edema in and about the neck.

When an aryl group is added to an active alkyl amine, as in benzylamine, the new compound retains the activity of the old (*F* in illustration). In this instance the benzene group replaces hydrogen on the carbon of the alkyl group. If it is tied to the nitrogen of the amine group (to yield the chemical isomer, toluidine) this activity practically disappears. The same is true of that other chemical isomer methyl-aniline.

We have also experimented with a series of nitrogenous compounds, other than those already mentioned, which are of interest because of their chemical relation to these compounds or because they are derived from the chemical nucleus of toxins, various protein-split products, including the ptomaines, or alkaloids. Thus we found pyridine (*G* in illustration), quinoline, creatinine and hexamethylenetetramine to produce swelling and solution of casein. Uric, hippuric and barbituric acids, on the other hand, were found to be without effect, as were also creatine hydrate, pyrrole, l-prolin, acetoxime, ethyl nitrite and ethyl nitrate.

#### COMMENT

In an attempt to explain what an acid or an alkali does to a protein to make it swell or dissolve, it was early pointed out that this has nothing to do with the hydrogen or hydroxyl ion concentrations observable in



the protein-water mixtures. The effect produced is dependent on compound formation. The proteins behave as do water-insoluble aminofatty acids or water-insoluble fatty acids. When on treatment with an alkali, for example, the latter are made to absorb more water or to pass into solution, one says not that this is because of some mysterious change in hydrogen or hydroxyl ion concentration but that the fatty acids unite with the alkali to yield a soap. Similarly, the aminofatty acids in uniting with alkali yield a soaplike compound. In both instances the newly formed products differ from the original aminofatty acid or the fatty acid in two ways—they have a greater capacity *for* dissolving water (swelling) and a greater solubility *in* water. The same action and the same effects may be observed on neutral protein when treated with alkali (as illustrated in the description of the action of ammonia on casein). We believe that the action of the amines here discussed is similar. They too, combine with the casein to yield a series of chemical derivatives which have a greater hydration capacity and a greater solubility in water. On this account they swell, become optically more homogeneous, soften or even liquefy.

We wish now to utilize these colloid-chemical findings in an attempt to make more precise the mechanism and the chemistry of that effect of injury which in physiology is expressed by the term “reaction to injury” and in pathology, by “inflammation.” To say that the product of such injury consists of swelling, cloudy swelling, softening, liquefaction, leukocytic infiltration and recovery, absorption or necrosis is to approach such an analysis, but it is an analysis still in the terms of biology. A more finite explanation would lie in the substitution of physical or chemical concepts for these terms.

The essence of inflammation receives a different definition depending on whether one asks a general<sup>9</sup> or a special<sup>10</sup> pathologist; and even in the latter case it makes a difference whether the answer is made from observation on avascular or on vascular tissue. Of the total set of changes marking the reaction to injury in vascular tissue several disappear as soon as nonvascular areas or plant structures are involved, for example, changes within blood vessels, increased redness and increased heat (the fraction due to the increased circulation of blood in a warm-blooded animal). Leukocytic infiltration obviously disappears in organisms that have no leukocytes and in plants. Pain is a criterion only in sensible organisms. Yet the morphologic essence of the reaction to injury persists throughout the organized world, for, as Kite<sup>11</sup> showed clearly, even the individual cell when stabbed with a glass needle shows

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9. Ransom, W. H.: *The Inflammation Idea in General Pathology*, London, Williams & Norgate, 1905.

10. Adami, J. G.: *Inflammation*, New York, The Macmillan Company, 1907.

11. Kite, G. L.: *Biol. Bull.* **25**:5, 1913; *Am. J. Physiol.* **32**:146, 1913.

a streak of swelling along the path of injury. In other words, the only universally present manifestations of the inflammatory response are swelling, cloudy swelling and softening or liquefaction of the involved part, to be followed by a reversal of these changes (in other words, recovery) or absorption, with or without intervening necrosis. The physiologic element of disturbed function appears both in avascular and in vascular regions. What needs emphasis is that it appears in the latter before any of the definitely vascular reactions of inflammation have taken place and that it may show itself in the avascular tissue of animals or in plants before any morphologic change is discoverable. An organ (such as any of the secreting glands or a muscle) may, in fact, be functionally dead without macroscopic or microscopic alteration. Put another way, a tissue may have lost all its functional capacity without evidence of any more visible change than is covered by the items of swelling, or cloudy swelling, with softening and liquefaction.

In the experiments detailed we have tried to indicate the chemical nature of these changes. They show how materials (other than acids) produced in the death of tissues or through the action of micro-organisms on living tissues act directly on proteins (in our experiments on the globulin half of the total proteins found in all cells) to alter their character in the direction of (*a*) swelling and (*b*) softening, liquefaction and solution.

Every attempt to analyze in chemical terms the effects of mechanical, thermal or electrical injury, or those of injury through parasitism, shows that materials of an amine nature are produced. This is true not only of the toxins (which are kept in a separate group not so much because of a biologic difference in poisonous effect as because of the type of chemical carrier to which they are bound) but especially of the toxic products of protein cleavage. Beginning with the bases produced in protein putrefaction and called ptomaines by Selmi, it was not long before Nencki isolated phenylethylamine from the group and Brieger, a whole set of mono-amines and diamines. This author achieved also the isolation of choline (hydroxyethyl trimethyl ammonium hydroxide, the degradation product of lecithin),<sup>12</sup> its next of kin, muscarine, and methylguanadine. More directly germane to this subject of the nature of the colloid-chemical changes observable in infection are such analyses as those of Emmerling.<sup>13</sup> From 860 Gm. of egg white inoculated with *Staphylococcus aureus* (besides more than 16.7 Gm. of various acids) he isolated 3.5 Gm. of trimethylamine; and from 600 Gm. of moist wheat gluten inoculated with *Proteus vulgaris*, 5 Gm. of ammonia and "appreciable quantities" of trimethylamine. This high ammonia value is largely

12. The colloid-chemical activity of the closely related tetramethylammonium hydroxide should be recalled.

13. Emmerling, O.: Ber. d. deutsch. chem. Gesellsch. **29**:2721, 1896.

the analytic product of amines. More recently Koessler and Hanke<sup>14</sup> proved the direct production of histamine from histidine in selected cultures of the colon bacillus. Amplification of the earlier studies of the chemical effects of sterile injury to tissue or of putrefaction or infection was brought by Vaughan and Novy<sup>15</sup> and Vaughan, Vaughan and Vaughan.<sup>16</sup> About the same time Eustis<sup>17</sup> showed the application of histamine (beta-iminazolyethylamine) to the slightly broken skin to be followed by intense urticaria, and Johnston<sup>18</sup> demonstrated the edema-producing effects of this compound and of various other amines on different tissues directly. Within recent years various amines have been discovered as the active producers of edema in sterile "wound" shock, in eclampsia and in several types of human infection—in short, in a whole series of pathologic states of which edema is the sole morphologic attribute.

The experiments detailed show what is the point of attack of such compounds. They act on the protein of the living cell and, uniting with it, yield a protein derivative. It does not matter, of course, whether at the point of injury the poisonous agent is produced immediately, as the product of protoplasmic degradation, or mediately, through the action of a pathogenic organism. In either case the protein derivative has a solvent capacity greater than normal for water and in water, and the colloid-chemical and the clinical or postmortem terms that describe the newly formed system are covered by swelling, softening, liquefaction and solution.

#### SUMMARY

Quantitative experiments are described illustrating the effects of a long series of different amines on the swelling, softening and solution of a pure protein (casein). The effects are attributed to compound formation in consequence of which proteinates possessed of a higher degree of solvent power for water and in water are produced. The identical compounds, elaborated in consequence of injury and of infection, are held to act similarly on protoplasm, through which an explanation in colloid-chemical terms is found for the swelling, softening and liquefaction of tissue characteristic of various pathologic states.

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14. A number of papers by these authors appeared in the *Journal of Biological Chemistry* from 1919 to 1924, several of which deal specifically with the direct production of histamine from histidine (*J. Biol. Chem.* **39**:521 and 539, 1919; **50**:131, 1922; **59**:803, 1924).

15. Vaughan, V. C., and Novy, F. J.: *Ptomains, Leucomains, Toxins and Antitoxins*, Philadelphia, Lea Brothers & Co., 1896.

16. Vaughan, V. C.; Vaughan, V. C., Jr., and Vaughan, J. W.: *Protein Split Products in Relation to Immunity and Disease*, Philadelphia, Lea & Febiger, 1913.

17. Eustis, Allan: *Am. J. M. Sc.* **143**:862, 1912; *New Orleans M. & S. J.* **66**:730, 1914.

18. Johnston, A. R.: *J. Infect. Dis.* **42**:473, 1928.

# CHANGES IN THE MYOCARDIUM OF RABBITS FROM AUGMENTING THE HEART RATE MECHANICALLY AND FROM INDUCED HYPERTHYROIDISM

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In a previous experimental study of the heart in hyperthyroidism<sup>1</sup> we discussed the various opinions concerning the lesions in human hearts in association with hyperthyroidism as well as the experimental evidence of the character of such pathologic disturbances in the hearts of animals used for experimental study. We concluded that the evidence of the existence of a specific thyroxine-induced cardiac lesion was not entirely convincing.

It was further suggested that such damage to the cardiac musculature might be the result of cardiac overwork resulting from hyperthyroidism rather than due to the direct impingement of thyroxine. This opinion was based on the occurrence of similar lesions in the heart of a rabbit in which the inhibitory nerve control to the heart was destroyed. This prompted us to investigate further the rôle of uncontrolled heart action in the production of the lesions in the heart in hyperthyroidism.

Accordingly, we selected a number of healthy rabbits, divided them into four groups and placed the animals in individual cages under clean hygienic conditions on a diet sufficient to maintain a relatively constant weight. Group 1 consisted of rabbits in which the depressor nervous mechanism of the heart was destroyed. Group 2 was composed of rabbits fed desiccated thyroid (Armour's) to the point of toxicity. Group 3 consisted of rabbits with the depressor mechanism destroyed, with the additional administration of ephedrine or desiccated thyroid or

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1. Menne, F. R.; Keane, R. H.; Henry, R. T., and Jones, N. W.: *Am. Heart J.* 8:75, 1932.

both. Group 4 consisted of rabbits under similar conditions of housing, but unmolested in any way. The following results were obtained:

#### EXPERIMENTS AND RESULTS

*Group 1.*—Ten rabbits were operated on under ether anesthesia. A number of these were given preliminary doses of sodium amytal but this was discontinued because of the undesirably long postoperative anesthesia resulting in respiratory congestion. The carotid canals were exposed through a midline incision, and an inch section of the depressor fibers on either side was removed. The carotid arteries were then carefully denuded of their investments up to and including the carotid sinus at the bifurcation forming the internal and external branches. Following this effacement, the denuded artery was gently swabbed with 95 per cent alcohol. An immediate enlargement of the arteries and an increase in the pulse rate were observed. Following the operation the animals were kept in a warming chamber until they completely recovered from the anesthesia.

All pulse rates were determined from the electrocardiographic tracings (lead II needles inserted beneath the skin were used for electrodes). A number of such tracings were made prior to operation in order to accustom the rabbits to being tied down and to determine the normal for a given rabbit. All such manipulation was accomplished with the *minimum excitement* in a quiet room. The normal pulse rate in this group varied from 175 to 330, the average being 251 per minute. The rabbits were kept under observation for a variable period from two to fifty-one days. Three of these animals showed a loss of weight; in two there existed a secondary infection. In seven of the ten rabbits an average increase in pulse rate of 63 beats per minute was obtained, while two actually showed a decreased pulse rate.

Although the electrocardiographic tracings were made with lead II, only certain changes were manifest in the action of the heart. There was evident a variation from simple tachycardia to inverted P and T waves with an occasionally altered QRS complex. In rabbit 29 there was an additional auricular fibrillation. These findings were not altogether constant or in proportion to the extent of the myocardial changes found at autopsy. They were, however, in general, suggestive of myocardial damage.

Seven of the rabbits were killed at the end of the observation period. Three were found dead. All were carefully studied at autopsy. Search was made for such disturbances as might have a bearing on the changes in the heart. In six of the rabbits, no noteworthy changes were found. In two there were stitch abscesses, while in two others abscesses were found in the neck and left lung, respectively.

*Gross Examination of the Hearts:* A moderate variation was observed in the gross appearance of the hearts (fig. 1). In general, however, the auricles were found moderately dilated. The subepicardial fat of the ventricles was scant. The left ventricles appeared elongated and hypertrophic. The hearts of the animals found dead were dilated, being filled with blood. The average weight of the hearts of the animals that were killed was 8.9 Gm. as compared with 12.2 Gm. in the rabbits that died, the difference being in part due to the blood content in the latter. The heaviest hearts were found in those animals in which infections were found and also in one in which there was an ileus due to cutting of the right vagus nerve. The smaller hearts were found in the rabbits that were observed the longest after operation.

The average length of the ventricles from the auriculoventricular groove to the apex was 3.4 cm., being the same in the animals that were killed and those that died. A variation occurred in the transverse diameters, the average in the animals that were killed being 2.44 cm. as compared with 2.76 cm. in those found

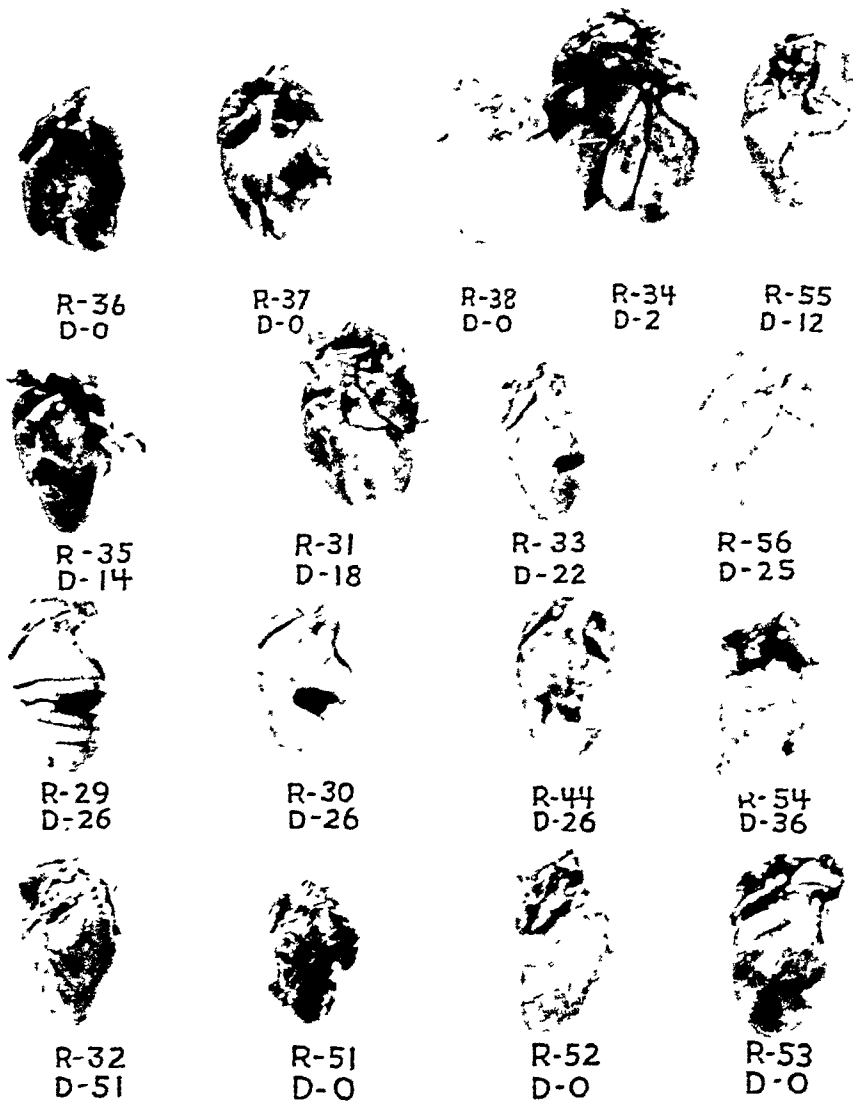


Fig. 1.—Photomicrograph showing the gross appearance of the hearts in group 1. Portions were excised promptly after weighing so that they might be fixed for the purpose of demonstrating glycogen.

dead. This difference was most marked at the apex (1 cm. from the tip) where the hearts of the animals that were killed measured 1.61 cm. as compared with 2.23 cm. in those that died. Evidently cardiac dilatation affected the transverse measurements to a greater extent than it did the lengths of the hearts. When the hearts were sectioned transversely, the reverse was found in the measurements of the left ventricles. In the animals that were killed the left ventricles averaged 1.8

cm. as compared with 1.6 cm. in the rabbits found dead. Hypertrophy of the wall was found to be responsible for this difference.

**Microscopic Examination:** Sections were made at three different levels through both ventricles. An attempt was made to determine the glycogen content by the use of special stains. Such efforts were unsuccessful. Because of an inability to demonstrate glycogen with such methods in control animals, the effort was abandoned. The glycogen in the cardiac muscle evidently exists in such a nebulous state that it is not readily detectable by staining methods. All sections were stained with hematoxylin and eosin and with phosphotungstic acid-hematoxylin. Relatively bizarre cytologic changes were observed. There was

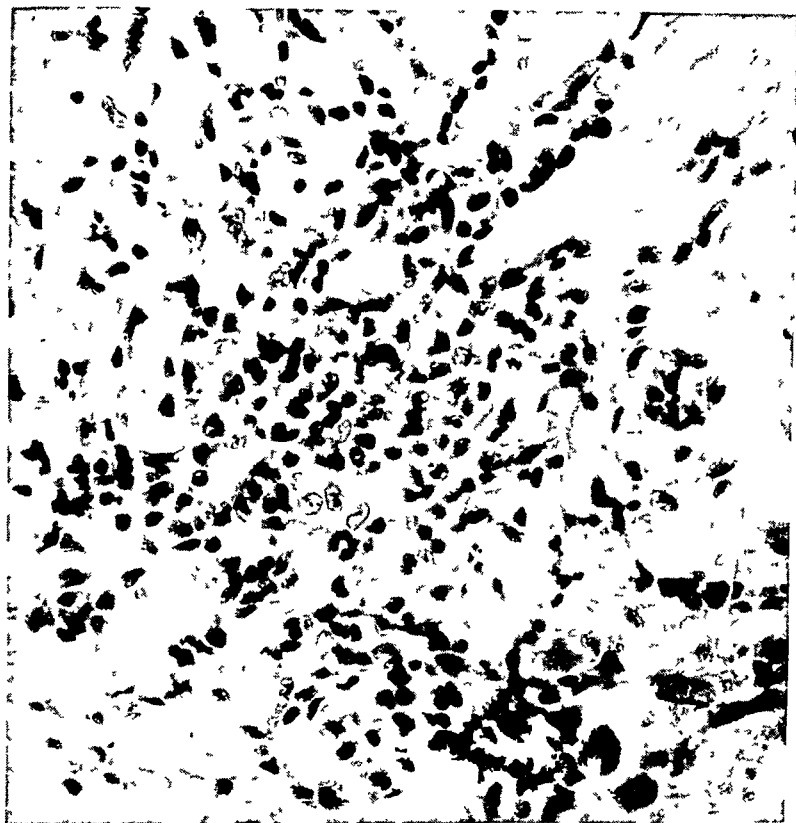


Fig. 2 (group 1).—Photomicrograph of an inflammatory-like intermuscular accumulation of histiocytes. There was no gross evidence of infection in this animal.

noticed an irregularity in staining. Even with the most careful technic certain areas failed to be differentiated. Here the muscle bundles appeared swollen, and the cross-striations were found to be blurred. Then, too, small collections of histiocytes (monocytes) would be seen to separate the muscle bundles (fig. 2). In still other areas distortion of muscle bundles with fraying, small fat vacuole formations and early hyperplasia of the fibrous connective tissue were noted (fig. 3), while in still other areas the fibrosis and wide separation of the muscle bundles were more extensive (figs. 4 and 5). These illustrations are all from the heart of rabbit 29. This animal was allowed to survive twenty-six days after operation. The course, with the exception of the increased heart rate and the general autopsy observations, was entirely normal. The lesions found in the

papillary muscles, particularly those supporting the chordae tendineae of the mitral valve, were found to be older, more hyaline and more extensive. On the basis of such abnormal histologic changes, examination of two of the hearts of the rabbits gave negative results while the results in five were considered slightly positive and in three definitely positive.

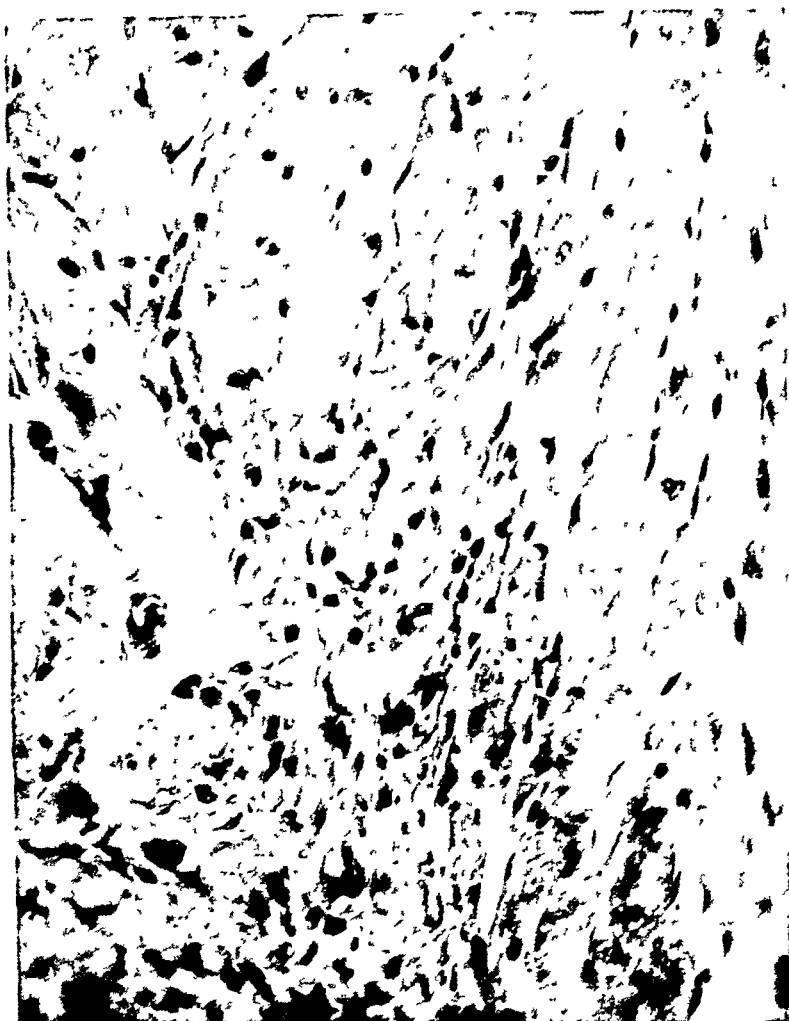


Fig. 3 (group 1).—Photomicrograph of an early focal degeneration of the myocardium. Note the vacuole formation and distortion of the continuity and relationship of the muscle bundles.

*Comment.*—In arriving at a conclusion as to the significance of the changes occurring in the hearts of rabbits with the depressor nerve mechanism to the heart destroyed, several important factors must be taken into consideration. First, the completeness of the operative intervention will be considered. Although it seems relatively simple, certain difficulties were encountered. The heart rate was, on the average, very rapid. In certain animals in which the heart rate was high, it was often



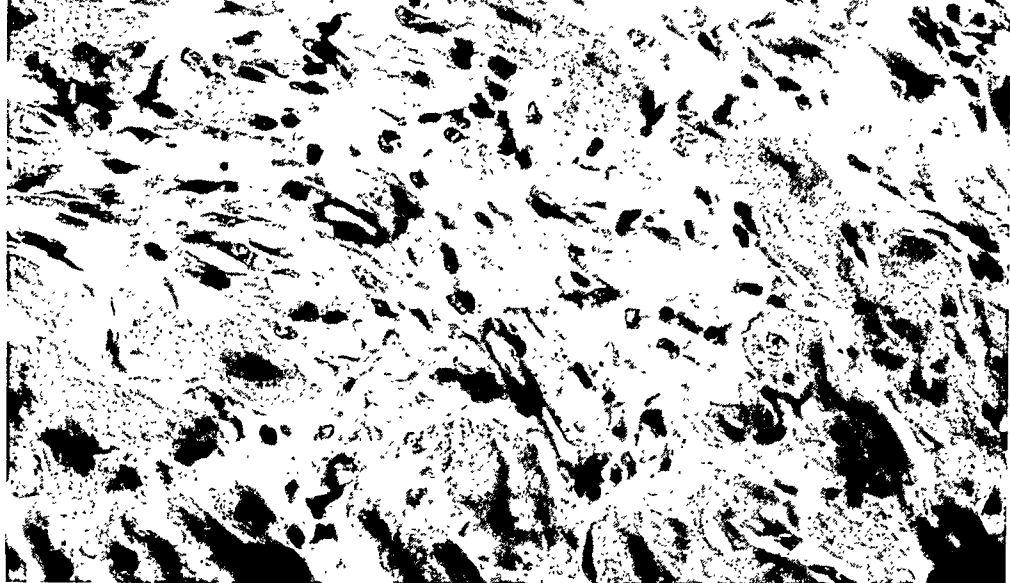


Fig. 4 (group 1).—Photomicrograph of a more advanced stage, with wider separation of muscle bundles and fraying, owing to focal fibrosis.

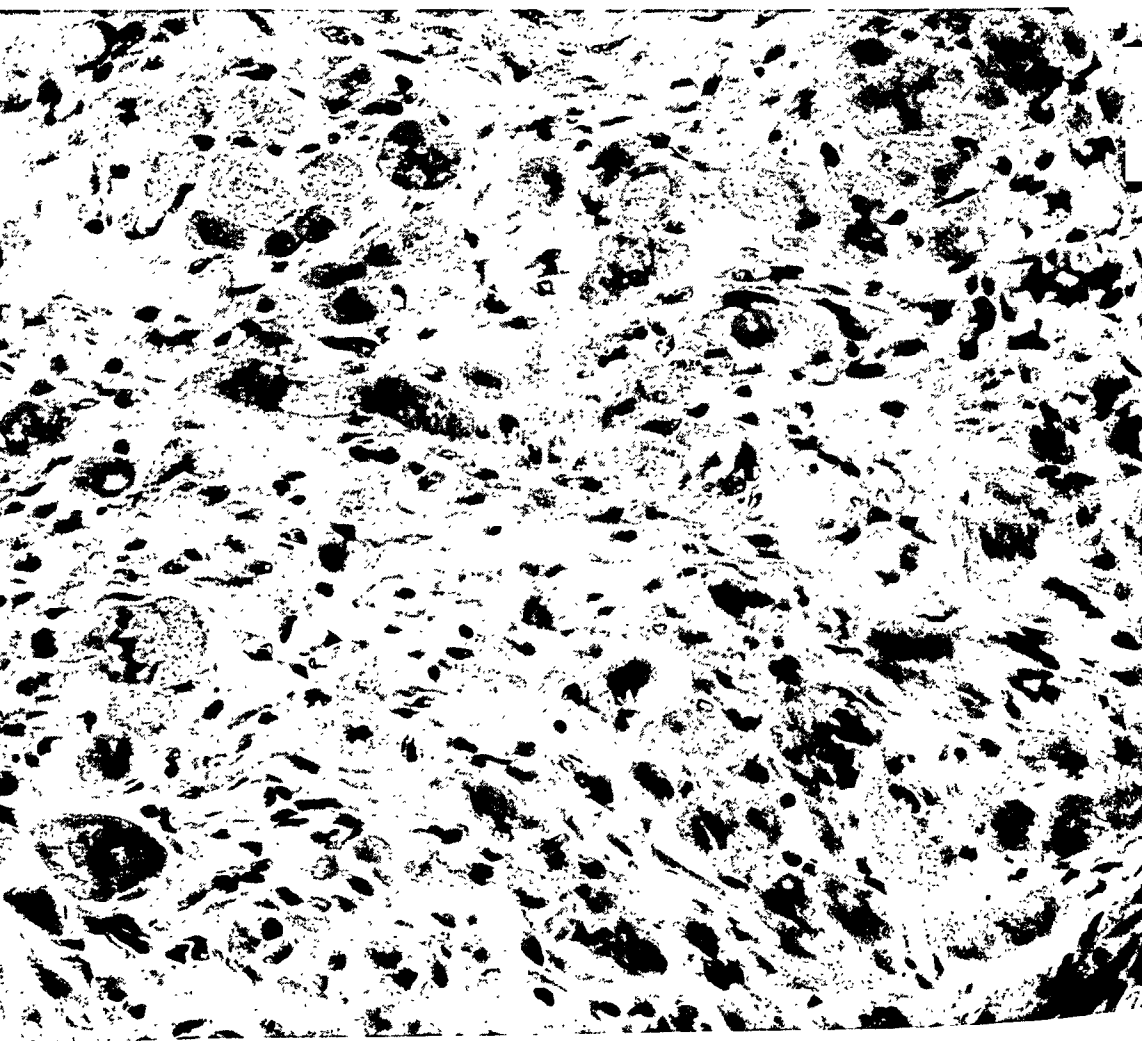


Fig. 5 (group 1).—Photomicrograph of an area of marked fibrous connective tissue separation and replacement of muscle bundles.

difficult to increase the rate by operation. Then, too, accessory anastomosing nerve fibers may and probably do occur, so that the inhibition was only in part destroyed, and this was apparently compensated for. In a number of instances, two or three depressor (?) fibers were found accompanying the vagus. Secondly, one must consider the possibility of individual variation in the compensatory adjustment of the cardiac activity, and thirdly, the fact that the animal was confined to its cage and not in its natural wide range habitat, where ordinary vicissitudes would influence the strain placed on its irritable heart, must be reckoned with. In addition to these the possibility of preexisting damage to the cardiac muscle must also be considered. There is also no way of determining many of the disturbances that may call for excessive cardiac strain such as occur in human beings. In this experiment there seems to be some relationship between the duration and the extent of the lesions. It would seem that they appeared most strikingly in the first three weeks, since one of the rabbits with a twenty-six day course showed more definite lesions than were found in the rabbit living fifty-one days after the operation.

There appears to be a parallelism of the increase of the heart rate and the occurrence of lesions within the heart. The more pronouncedly damaged hearts were found, with one exception (rabbit 30), in the rabbits in which the heart rate was increased above 60 beats per minute. Individual compensatory adjustment may account for the lack of exact gradation of the lesions. Most of these animals were observed to be inactive. They were found lying down a great deal of the time. This undoubtedly had the effect of lessening the strain on the heart. The temperament of the individual animals must be considered in allowing for such compensatory adjustments.

The rôle of infections in the production of the pathologic changes in the hearts is of interest, since it may be argued that they are the causative agents. Three of the rabbits in this series had infections. One of these died with a stitch abscess, but no lesions were found in the heart. In the other two, which had distinctive changes in the heart, such lesions were not considered more pronounced than those in the hearts of the animals that had no traceable infection. In none of the hearts of the infected animals was there evidence of mycotic foci. Polymorphonuclear leukocytic infiltration was not found, and yet one cannot exclude the possibility of circulating toxins in such instances. We were unable, therefore, to conclude that such lesions were produced by the coexisting infections, although we must admit their augmenting rôle.

There appears to us to be a definite relationship of the lesions in the hearts of the rabbits of this group to the altered increased rate of the heart induced by destroying as completely as possible the inhibitory nerve mechanism.

TABLE 1 (Group 1).—*Experimental Data Concerning Rabbits with Cardiac Inhibition Destroyed*

Experimental Data							Data on Heart					Results	Comment
Number	Weight, Kg.	Pulse	Num-ber of Days	Weight Loss, Kg.	Pulse In-crease	Death*	Weight, Gm.	Length, Cm.	Width at Base, Cm.	Width at Apex, Cm.	Width of Left Ven-tricle, Cm.		
29	2.39	240	26	0	60	A	8.2	4.0	2.5	1.6	1.7	Positive +++	Observation: Negative course Autopsy: Negative; heart: gross—pale, right auricle dilated, elongated; microscopic—atrophy, hyperten-sion, fibrosis, fraying
30	2.50	225	26	0	75	A	7.0	3.0	2.0	1.4	1.75	Positive +	Observation: Negative course Autopsy: Negative; heart: gross—broad at base, right chambers dilated, petechial hemorrhage in myocar-dium; microscopic—cell infiltration, fraying, atrophy, hypertension
31	2.16	240	18	0	45	D	15.9	3.5	2.8	2.5	1.7	Negative	Observation: Stitch abscess; died Autopsy: Abscess in neck; general parenchymatous degeneration; heart: gross—right auricle dilated; microscopic—parenchymatous degeneration, edema, fragmentation
32	2.39	240	51	0	48	A	8.7	3.2	2.3	1.5	1.8	Positive +	Observation: Negative course; left carotid artery ligated Autopsy: Negative; heart: gross—firm, moderately elongated, auricles dilated; microscopic—focal atrophy and hypertension, fatty degeneration, fraying, fibrosis
33	2.27	330	22	0	—30	A	5.7	3.1	2.0	1.4	1.5	Negative	Observation: Negative course Autopsy: Negative; heart: gross—atrophy, narrow, elongated; microscopic—focal atrophy and hyper-tension, hyaline degeneration, slight fibrosis

Observation: Negative course  
Autopsy: Negative; heart: gross—pale, right auricle dilated, elongated; microscopic—atrophy, hyperten-sion, fibrosis, fraying

Observation: Negative course  
Autopsy: Negative; heart: gross—broad at base, right chambers dilated, petechial hemorrhage in myocar-dium; microscopic—cell infiltration, fraying, atrophy, hypertension

Observation: Stitch abscess; died  
Autopsy: Abscess in neck; general parenchymatous degeneration; heart: gross—right auricle dilated; microscopic—parenchymatous degeneration, edema, fragmentation

Observation: Negative course; left carotid artery ligated  
Autopsy: Negative; heart: gross—firm, moderately elongated, auricles dilated; microscopic—focal atrophy and hypertension, fatty degeneration, fraying, fibrosis

Observation: Negative course  
Autopsy: Negative; heart: gross—atrophy, narrow, elongated; microscopic—focal atrophy and hyper-tension, hyaline degeneration, slight fibrosis

34	2.61	300	2	0	—42	D	11.0	3.8	3.2	2.5	1.8	Positive +	Observation: Right vagina cut at operation; spasmodic respiration; diarrhea; found dead Autopsy: Emphysema, congested pleural cavity, dilatation of stomach and bowel; heart: gross—marked dilatation; microscope—atrophy, fragmented, fraying, fibrosis, hyaline degeneration
35	2.30	210	14	0.56	60	D	9.7	2.9	2.3	1.7	1.3	Positive + + + +	Observation: Stillborn abscess; found dead Autopsy: Abscess in neck, congested pleural cavity; heart: gross dilatation, small at point of apex; microscope—atrophy, hypertension, fatty and hyaline degeneration, cellular infiltration, fibrosis, fraying
54	2.09	320	35	0	0	A	10.4	3.5	2.4	1.7	1.7	Positive +	Observation: Negative course; gave birth to 2 rabbits Autopsy: Negative; heart: gross elongated, hypertension, left ventricle pale; microscope—focal hypertension and atrophy, hyaline degeneration, fibrosis
55	3.80	200	12	0.53	60	A	10.85	3.4	2.9	1.9	2.1	Positive + +	Observation: Negative course Autopsy: Negative; heart: gross—dilated, broad base, pointed apex; microscope focal atrophy and hypertension, cellular infiltration, fraying, fibrosis, hyaline degeneration
56	3.87	175	25	0.30	80	A	11.7	3.6	3.0	1.8	2.1	Positive + + + +	Observation: Negative course Autopsy: Small abscess of left lower lobe; heart: gross—pale, broad base; microscope—focal edema and atrophy, fibrosis and fraying
Average	2.63	251	..	...	7R, 63 + 3R, 21 —	..	A, 8.9 D, 12.2	A, 3.4 D, 3.4	A, 2.44 D, 2.26	A, 1.61 D, 2.23	A, 1.8 D, 1.6	Positive 8 Negative 2	

\* In this and the following tables, A indicates animals killed, and D, those that died.

In order to study further the comparative effects of unbridled action of the heart, mechanically induced, with that of the rapid heart rate of rabbits with induced hyperthyroidism, we decided to investigate a somewhat similar series of such animals under the same conditions.

*Group 2*—The rabbits were about the same average weight as those in group 1. They were raised, housed and fed in the same manner. They were given doses of desiccated thyroid (Armour's) in amounts varying from 90 to

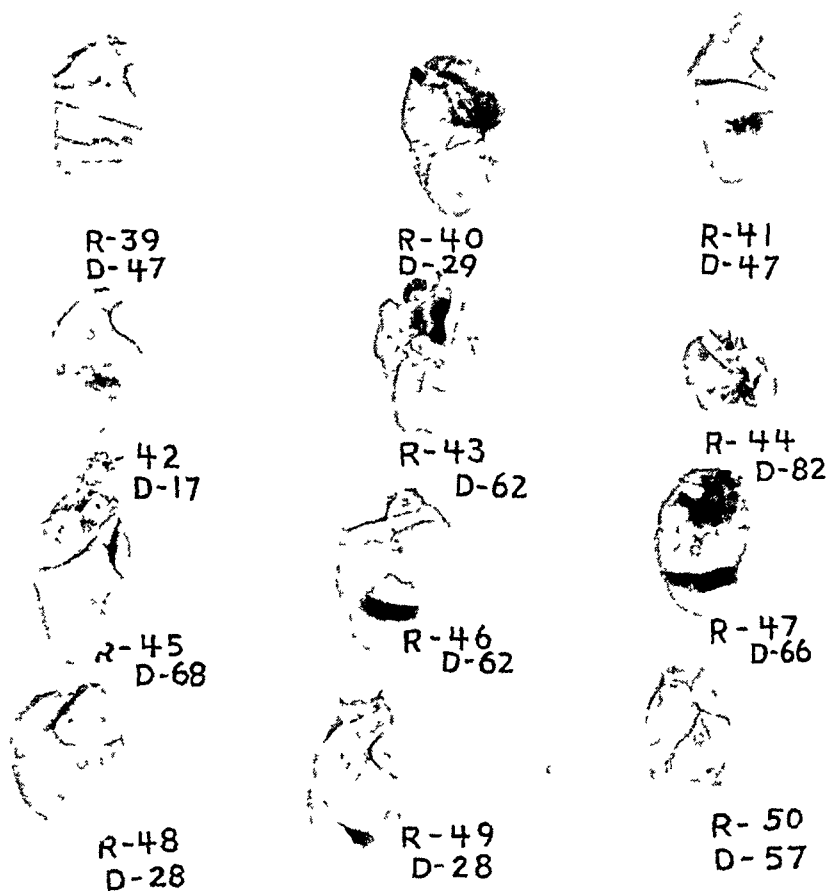


Fig. 6 (group 2).—Photograph of the gross appearance of the hearts of rabbits with induced hyperthyroidism

720 mg. daily in two dose-periods of varying days with various intermissions, the object of this procedure being to induce the animal to pass through two successive toxic periods. Considerable variation was encountered as to the tolerance of rabbits toward desiccated thyroid. Some of them became rapidly toxic on 90 mg of thyroid a day, while others increased in weight, became complacent and even somewhat lethargic. In four instances it became necessary to increase the dosage to from 270 to 720 mg. daily before the toxic symptoms began to appear. The toxic dosage was continued until marked loss of weight, increased irritability and weakness developed. It was then discontinued until the animals began to approach

a normal status, when a second series of feeding was started and carried to a similar end-point. Each animal, therefore, received two rather distinctive jolts.

The average increase in pulse rate (taken by electrocardiographic tracings) ranged from 30 to 165 beats per minute, the average for the group being 82. The

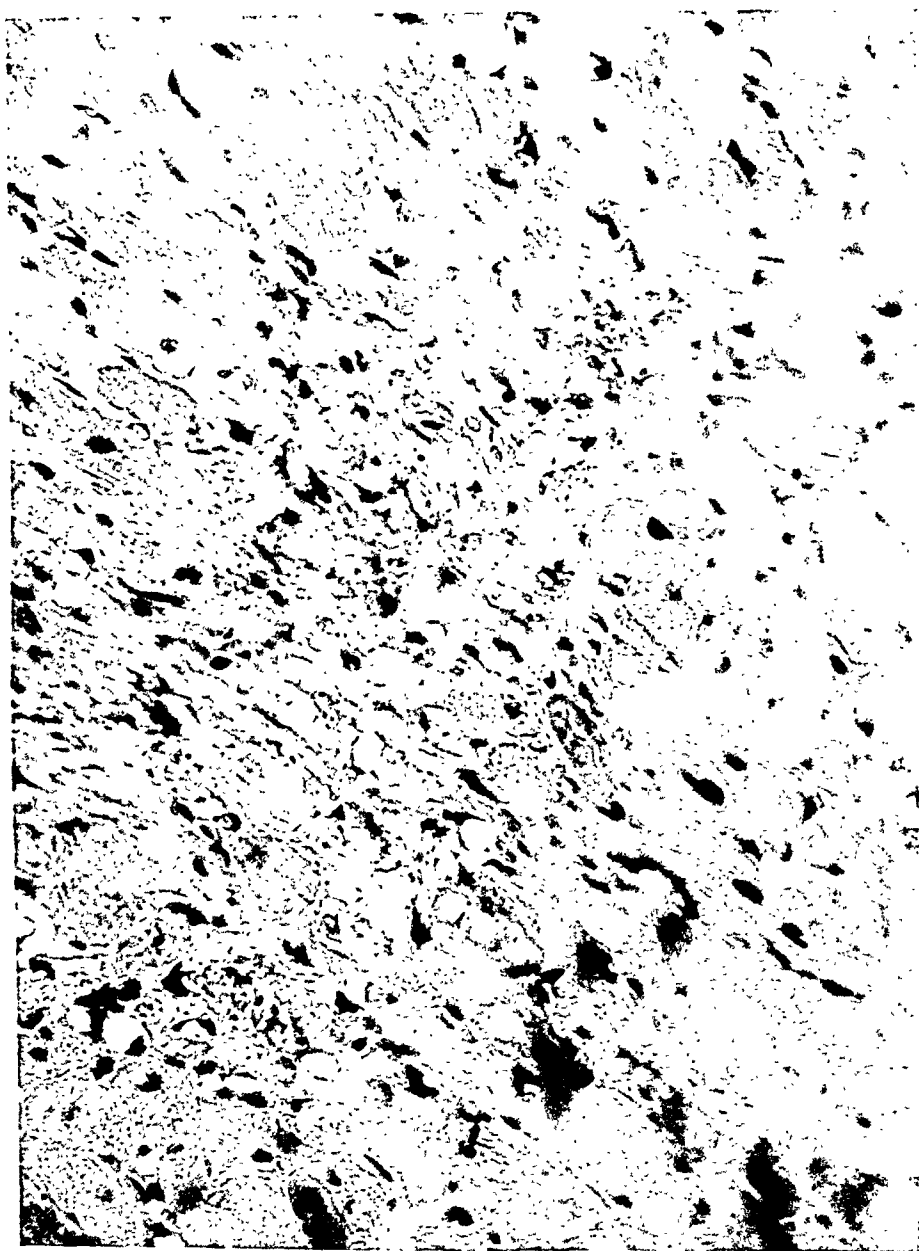


Fig. 7 (group 2).—Photomicrograph of the myocardial damage with fat vacuole formation, fraying of muscle bundles and early fibrosis.

majority of the rates were high, since the average also includes those slower rates occurring when the animal was approaching collapse.

The electrocardiographic tracings (lead II) revealed a marked variation from inverted P and T waves to regular or slower or normal rhythm, while in others the reverse was true. Rabbit 50 showed an inverted P wave occurring at irregular

times. In rabbit 48 there was a regular to irregular rhythm with sinus block. In rabbit 43 the rhythm was regular to irregular with final flutter. The latter occurred in three rabbits (43, 46 and 47). These changes also point to myocardial damage. Again the variations in rhythm were not entirely consistent with the modifications of the cardiac muscle found at autopsy. Six of these rabbits were killed when they were near collapse, while five died during the night.

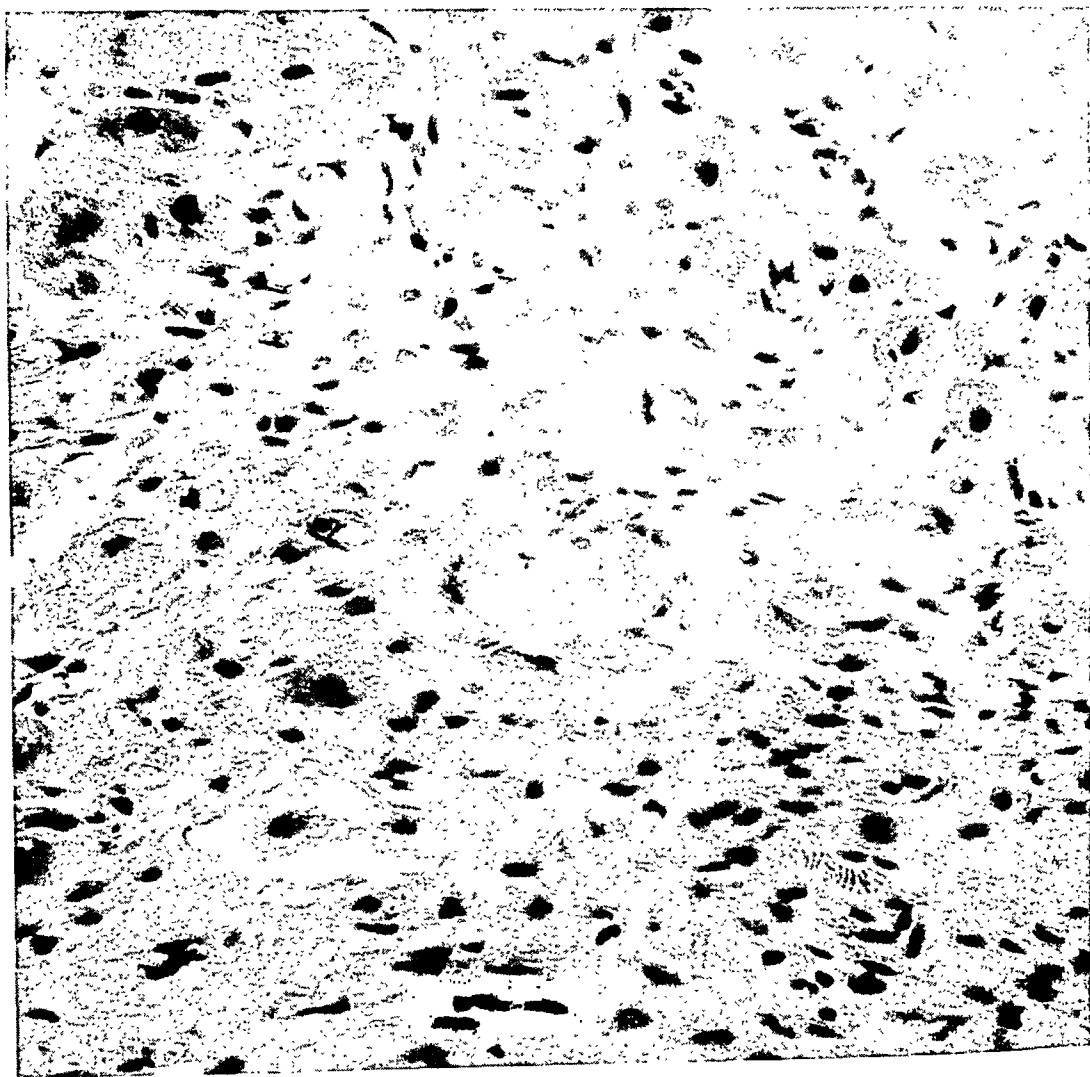


Fig. 8 (group 2).—Photomicrograph showing extensive degeneration and separation of the muscle bundles with fibrous connective tissue replacement.

Autopsy revealed general emaciation in all, and in two (43 and 48) abscesses were found in the right pleural cavities. No other noteworthy gross pathologic changes were found.

Gross Examination of the Hearts: All of the hearts were practically devoid of subepicardial fat (fig. 6). Four of the five hearts of the rabbits that were found dead showed marked right-sided dilatation. In general, the hearts of the rabbits that were killed were pale, firm and elongated, the average weight being 8.16 Gm. As may be seen in table 2, all measurements were reduced, with such variations as might be expected in dilated hearts.

**Microscopic Examination:** As was true in the examination of the hearts in group 1, a similar but more extensive polychromatophilia was noted. Regional shrinking and focal hypertrophy of the muscle bundles were observed. A spotty swelling of the muscle bundles with blurring of the cross-striation was noted. In still other areas rather complete dissociation with interruption of the muscle bundles was seen (fig. 7). Here a fibrillar reticulum separated by circular fat vacuoles with fraying of the muscle bundles and early proliferation of fibroblasts was noted. As in the examination of the hearts in group 1, all gradations to larger areas of replacement of fibrous connective tissue were observed (fig. 8). Here, too, the pathologic lesions were found in the left ventricle. They appeared to be more pronounced in the papillary muscles and in the lower half of the left ventricular walls. Ten of these hearts were regarded as having definite lesions. The latter were most pronounced in the hearts of the rabbits with abscesses in the pleural cavities.

*Comment.*—It seems to us that there is sufficient evidence to indicate that distinctive damage to cardiac musculature occurred in these rabbits following the administration of repeated doses of desiccated thyroid. While it is true that heroic doses were administered over long periods in order to produce such disturbances, it must be remembered that the housing of the rabbits in cages is comparable to placing a patient with hyperthyroidism at rest in bed. Under such conditions there is a minimum taxation of cardiac function. The variation in the dosage employed and the total number of days required as well as the time interval between doses appear to us to be consistent with individual constitutional ability of each animal to resist, maintain and restore metabolic equilibrium. Intimately linked with this status are the loss of weight, increase in the pulse rate and the ability to resist infection and death. The weights and measurements of all the hearts in this group are proportionate to the variations in body weight, the terminal condition of the body as a whole and of the heart in particular being one of inanition. The variations in the extent of cardiac damage, as may be seen in table 2, were not always proportionate to the severity of the toxicity, since the animals that were found dead from the thyroid insult did not always reveal extensive pathologic changes in the heart. This fact may mean that our examination and criteria of cardiac disturbances were not sufficiently comprehensive or that greater influence must be attached to extracardiac failure in bringing about death. It is likely that rabbit hearts may vary in their susceptibility to injurious agents just as do human hearts. In rabbits 43 and 48, the presence of an infection (probably secondary to the lowered resistance) seems to have augmented the destruction of the cardiac muscle. There was no way of determining the exact relationship of these two conditions in these animals. The fact that there were these discrepancies in results when the body was flooded with the active principle of desiccated thyroid would seem to vitiate the contention that there is a specific differentiated effect of thyroxine on the cardiac muscle.



TABLE 2 (Group 2).—*Effect of Feeding Desiccated Thyroid to Rabbits*

Experimental Data										Data on Heart					Results	Comment
Num- ber	Weight, Kg.	Mg. Thyroid Daily	Num- ber of Days	Inter- val, Days	Amount of Thyroid Fed, Gm.	Weight, Loss, Kg.	Pulse In- crease	Death	Weight, Gm.	Length, Cm.	Width at		Ven- tricle, Cm.			
											Base, Cm.	Apex, Cm.				
50	2.12	186	90-180	56	15	7.56	1.0	72	A	9.9	3.9	2.2	1.7	1.5	Pos. +++	Observation: Irritability, loss of weight, diarrhea, weakness Autopsy: Emaciation, atrophy; heart: gross—small, shrunken, broad at base; microscopic—atrophy, focal hypertension, regeneration, fragmentation, fatty changes, fraying, fibrosis
47	2.82	180	90-180	65	15	9.18	0.97	76	A	9.5	3.3	2.5	1.9	1.7	Pos. +	Observation: Irritability, weakness, prostration Autopsy: Emaciation, congested pleural cavity; heart: gross—negative; microscopic—polychromatophilia, edema, fraying, slight fibrosis
48	2.57	300	90-180	65	15	9.18	0.94	82	D	9.9	3.5	2.8	2.6	1.8	Pos. ++++	Observation: Marked toxicity, dropped heart bent; found dead Autopsy: Abscess, right pleural cavity, extending through diaphragm; heart: gross—firm, elongated; microscopic—marked chromato- philia, fibrosis, fraying, edema, fatty change
49	2.61	155	90-180	66	15	9.36	0.46	100	A	8.45	3.2	2.5	2.2	2.0	Pos. +	Observation: Emaciation, diarrhea, weight in- crease during intermission Autopsy: Negative; heart: gross—small, irri- table; microscopic — polychromatophilia, atrophy, hypertrophy, fatty changes, fibrosis
42	2.21	150	180	17	0	3.06	0.42	90	A	6.9	3.1	2.3	1.6	1.5	Neg.	Observation: Irritability, emaciation, exhaus- tion Autopsy: Negative; heart: gross—small, pale, mottled, firm; microscopic—marked polychro- matophilia, focal atrophy and hypertrophy

40	2.65	210	180	17	13	3.06	0.32	70	D	11.0	3.5	2.4	1.9	1.7	Pos. +	Observation: Loss of weight, died suddenly Autopsy: Negative; heart: gross—slight hemato-pericarditis, dilated; microscope—polychromatophilia, focal atrophy and hypertension, slight focal fatty degeneration, fibrosis, fragmentation, fraying
39	2.61	240	180-270	47	26	9.63	0.69	60	A	7.0	3.6	2.3	1.3	1.7	Pos. +	Observation: Extreme irritability, weakness, diarrhea Autopsy: Marked emaciation; heart: gross—small, firm, left ventricle eccentric; microscope—polychromatophilia, focal atrophy and hypertension, fragmentation
41	2.62	210	180-360	47	26	13.86	1.23	90	A	7.2	3.5	2.3	1.80	1.6	Pos. +	Observation: Increasing tolerance, final irritability, diarrhea Autopsy: Marked emaciation, congested pleural cavity; heart: gross—elongated, pale, firm; microscope — polychromatophilia, focal atrophy and hypertension, hyaline degeneration, edema, fragmentation
46	2.27	155	180	62	9	11.16	0.91	165	D	11.47	3.6	2.7	2.0	1.7	Pos. ++	Observation: Irritability, exhaustion, found dead Autopsy: Negative; heart: gross — dilated, pointed apex; microscope—polychromatophilia, hyaline degeneration, edema, fragmentation, fibrosis
45	2.40	285	180-270	34	9	13.59	0.61	65	D	12.8	3.4	2.3	1.6	1.7	Pos. ++	Observation: Irritability, loss of weight; died during intermission Autopsy: Congested pleural cavity; heart: gross — elongated, firm, pointed; microscope—polychromatophilia, focal atrophy and hypertension, hyaline degeneration, cellular infiltration, fibrosis
43	2.12	210	180-720	62	26	22.11	0.72	30	D	10.0	3.1	2.6	2.1	1.6	Pos. ++ + L	Observation: Increasing tolerance, weight increased, final diarrhea, weakness, prostration; found dead Autopsy: Congested pleural cavity, abscess of lower lobe of right lung; heart: gross—pale, dilated; microscope—parenchymatous degeneration and hypertension, edema, marked fibrosis
Aver.	2.4	207.3	.....	..	..	.....	0.75	S2	6, A 5, D	A, 8.16 D, 11.03	A, 3.83 D, 3.48	A, 2.35 D, 2.36	A, 1.75 D, 1.65	A, 1.63 D, 1.70	Pos., 10 Neg., 1	

TABLE 3).—*Experimental Data Concerning Rabbits with Cardiac Inhibition Destroyed Followed by the Administration of Desiccated Thyroid and Ephedrine*

Subgroup A					Data on Heart							Result	Comment	
Num- ber	Weight, Kg.	Pulse	Treatment	Num- ber of Days	Loss, Kg.	Pulse Increase mm. Hg. to —50	Pulse Increase mm. Hg. Death	Weight, Gm.	Length, Cm.	Width at Base, Cm.	Width at Apex, Cm.			Width of Left Ven- tricle, Cm.
44	2.05	200	Desiccated thyroid, 180 mg., 23 days post- operatively	44	0.16	+50 to —50	A	8.75	3.2	2.5	1.9	1.8	++++	Observation: Weight decreased, weakness, diarrhea, following thyroid administration Autopsy: Chronic fibrous pleuritis on left side, congested pleural cavity; heart: gross—elongated, pale, firm; microscopic—marked fatty and hyaline degeneration, cellular infiltration, fraying, fibrosis
65	3.12	256	Desiccated thyroid, 180 mg.; ephedrine, 3 mg. daily	25 (11)	0.88	45.0	A	8.9	3.5	2.7	1.7	1.9	++++	Observation: Loss of weight, weak- ness, exhaustion, following thy- roid administration Autopsy: Negative; heart: gross— hypertrophied left ventricle, pale, mottled; microscopic—hyperten- sion, cellular infiltration, fatty and hyaline degeneration, fibrosis, fraying
67	3.0	271	Desiccated thyroid, 180 mg.; ephedrine, 3 mg. daily	25 (11)	1.64	77.5	D	6.02	2.9	2.5	1.7	1.8	+	Observation: Emaciation, weak- ness, prostration Autopsy: Negative; heart: gross— small and firm; microscopic— atrophy, edema, hyaline and fatty degeneration, focal fibrosis
Aver.	2.72	242			0.89	40.8		A, 8.82 D, 6.02	A, 3.35 D, 2.9	A, 2.6 D, 2.5	A, 1.8 D, 1.7	A, 1.7 D, 1.8		
61	3.9	200	Ephedrine, 4 mg. twice a day	Subgroup B (47) 56	0.3	32.7	A	10.2	2.7	2.6	1.8	2.0	++	Observation: Negative course Autopsy: Negative; heart: gross— large, hypertrophied; microscopic hypertension, cellular infiltration, fatty and hyaline degeneration, fibrosis
62	3.3	220	Ephedrine, 4 mg. twice a day	(19) 35	0	86.5	A	11.5	2.7	2.7	2.0	1.9	+	Observation: Negative course Autopsy: Negative; heart: gross— hypertrophied; microscopic— slight atrophy, hypertension, fatty degeneration, cellular infiltration, fibrosis
63	2.1	228	Ephedrine, 4 mg. twice a day	(28) 42	0.1	72.5	A	8.77	2.7	2.9	1.9	2.2	—	Observation: Negative course Autopsy: Negative; heart: gross— enlarged; microscopic—negative except for hypertension
64	3.5	248	Ephedrine, 4 mg. twice a day	(26) 39	0.2	20.1	A	10.0	3.1	3.0	2.0	2.0	+++	Observation: Negative course Autopsy: Negative; heart: gross— left ventricle hypertrophied, dilated; microscopic—focal atrophy and hypertension, hya- line degeneration, fibrosis, fray- ing, edema
Aver.	3.2	224			0.15	36.6		9.1	2.8	2.8	1.9	2.0		

*Group 3* (table 3).—Since we were able to produce comparable cardiac lesions by either of the two previous experimental procedures (groups 1 and 2), it occurred to us that a combination of both of these experimental conditions should be productive of cumulative results. We therefore operated on seven rabbits. After their immediate recovery from the operation (forty-eight hours), desiccated thyroid was administered to three in daily doses of 180 mg., while two rabbits were given in addition daily intramuscular injections of 4 mg. of ephedrine. In addition, four rabbits were given 4 mg. of ephedrine twice a day but were not fed desiccated thyroid. The ephedrine was added in an attempt to increase the peripheral resistance by raising the pulse pressure and thereby increasing the work of the heart. The pulse rates and blood pressures were determined by the method of MacGregor<sup>2</sup> before each injection of ephedrine. The foregoing dosage was found to bring about the maximum increase in the pulse pressure in the different animals used. Although the series is small, there was found a decided variation in the response to ephedrine. The ephedrine was not administered during the entire period of observation, but only for the number of days indicated in the brackets ("No. of days," table 3).

In the animals fed desiccated thyroid, the loss in weight was found proportionate to the degree of toxicity. The increase in pulse rate was found greatest in the two rabbits receiving both desiccated thyroid and ephedrine. Although the average increase was not as great in any of these animals as it was in those of groups 2 and 3, the counter-effect of the ephedrine may have been responsible. The increase in pulse pressure was apparently somewhat greater in the rabbits (65 and 67) that received both desiccated thyroid and ephedrine. Only one of these animals (67) died of prostration.

The clinical course of these animals is in sharp contrast in the two subgroups. After several days the rabbits given desiccated thyroid became irritable, lost weight and became weak, diarrhea and prostration appearing, while the course of the rabbits receiving only ephedrine was negative.

The electrocardiographic tracings (lead II) revealed simple tachycardia in two of the rabbits (61 and 65), while in rabbit 44 there were inverted P and T waves with irregular cycle lengths indicative of dying muscle. In rabbit 44 there was also a tachycardia with inverted P and T waves with cycles of flutter. Only two tracings were made of these animals, but the findings were consistent with the damage in the hearts.

The autopsies in general gave negative results, with the exception of the occurrence of an old chronic fibrous pleuritis in one rabbit (44).

**Gross Examination of the Hearts:** The hearts in rabbits 44, 65 and 67 were small, pale and devoid of subepicardial fat and averaged 7.42 Gm. in weight. On the other hand, the hearts of the group of rabbits that received ephedrine were large, broad, deeply pinkish red and covered with subepicardial fat, and averaged 10.1 Gm. in weight. As is indicated in the measurements, the hearts in subgroup A were longer and narrower than those in subgroup B. In the rabbits receiving postoperative toxic doses of desiccated thyroid, a badly damaged cardiac muscle (4 plus) developed, while a similar effect was produced in only one of the two rabbits receiving in addition maximum doses of ephedrine for the same period; this variation is unexplainable, since in the second animal (67) a greater loss in weight and increase in pulse rate and pulse pressure developed, unless individual resistance is considered responsible. The results obtained in the rabbit receiv-

2. MacGregor, Leone: Arch. Path. 5:630, 1928.

TABLE 4 (Group 4).—*Data on Controls—Normal Rabbits*

Number	Weight, Kg.	Pulse	Number of Days	Weight Loss, Kg.	Pulse Increase	Death	Weight, Gm.	Length, Cm.	Data on Heart			Result	Comment
									Width at Base, Cm.	Width at Apex, Cm.	Width of Left Ventricle, Cm.		
36	2.39	180	25	0.09	0	D	8.5	3.2	2.6	1.9	1.7	Neg.	Observation: Negative course; died at operation Autopsy: Negative; heart: gross—slightly dilated; microscopic—negative
37	2.39	240	25	0	0	D	10.1	2.9	2.7	1.6	1.7	Neg.	Observation: Negative course; died at operation Autopsy: Negative; heart: gross—dilatation; microscopic—negative
38	2.16	210	25	0.11	0	D	7.7	2.4	2.6	2.0	1.8	Neg.	Observation: Negative course; died at operation Autopsy: Negative; heart: gross—dilatation, pale; microscopic—edema, negative otherwise
51	2.20	210	68	0	0	A	7.2	2.9	2.3	1.3	1.8	Neg.	Observation: Pulse irregularity with extrasystole (constant) probable sinus block Autopsy: Negative; heart: gross—negative; microscopic, negative
52	1.52	180	68	+1.59	0	A	8.7	3.1	2.5	1.2	1.1	Neg.	Observation: Negative course Autopsy: Negative; heart: gross—pale, pointed apex, broad at base; microscopic—negative
53	2.29	180	68	+1.07	0	A	10.9	3.4	2.6	1.8	1.9	Neg.	Observation: Negative course Autopsy: Negative; heart: gross—negative; microscopic, negative
Average	2.16	200	..	....	0	..	D, 8.84 A, 8.93	D, 2.85 A, 3.13	D, 2.63 A, 2.46	D, 1.83 A, 1.43	D, 1.73 A, 1.6		

ing only ephedrine following the operation were definite, but the lesions in the heart were not as extensive as anticipated. In addition to technical difficulties, the probable preoperative hypertrophy of these hearts must be considered as mitigating the effect, since the ephedrine was administered from nineteen to forty-seven days prior to the destruction of the inhibitory mechanisms of the hearts.

Apart from these modifying influences this experiment suggests an augmented supportive rôle of destroyed cardiac nerve inhibition, the administration of desiccated thyroid and the increase of vascular tonus.

*Group 4 (table 4).*—This group of six rabbits constitute the normal control group. These rabbits were selected and kept under the same conditions as those in the other experimental series. They remained at a relatively constant weight except for an increase in two of them. Three died at operation. Their pulse rates remained relatively constant, averaging 200 beats per minute. One of the rabbits revealed a cardiac irregularity interpreted as a sinus block, extrasystoles appearing in the electrocardiographic tracings. Rabbits 36, 37 and 38 continued to have a normal rhythm. In rabbits 52 and 53 there were inverted P and T waves. The clinical course was otherwise negative as were the gross findings at autopsy.

*Gross Examination of the Hearts:* All of the hearts were considered of moderate size and rather deeply colored, with the usual distribution of subepicardial fat. Except in two instances (37 and 53), the weights were relatively constant, averaging about 8.8 Gm. The hearts of the rabbits that died were shorter and broader than those of the ones that were killed.

*Microscopic Examination:* None of the lesions described in the hearts of the other groups were seen.

#### COMPARATIVE RESULTS

A consideration of the comparative results (table 5) revealed a relative uniformity in the average original weights of the rabbits used in the different series, the one exception being those in subgroup A in group 3. There was no loss of weight in group 1, the rabbits operated on to destroy the inhibitory fibers to the heart. The greatest loss in weight occurred in the series of group 3 A, in which both the operative procedure and the administration of desiccated thyroid were used. Considering the variations in pulse rate that may be found in normal rabbits, the original average pulse rates were fairly uniform (200 to 251). The greatest increase in pulse rate occurred in rabbits fed desiccated thyroid (increase of 82 per minute). Comparable with this is the increase in pulse rate of 63 in seven of the rabbits of group 1, while in three of the rabbits decreased pulse rates actually developed. In these animals the pulse rate decreased immediately following the operation, but showed an increase later in the course of observation.

Certain irregularities occur in the weights of the hearts. Whenever autopsy was performed the large vessels leaving and entering the hearts were promptly cut so that the heart that stopped in systole could be emptied of blood before it was accurately weighed. This was not possible in the animals found dead (having died during the night). For

TABLE 5.—Comparative Results

Group Rabbits		Average Weight, Loss, Kg.	Average Weight, Kg.	Pulse Rate	Average Pulse Variation	Death	Data on Heart												Results	
							Weight		Length		Width at Base		Width at Apex		Width at Left Ventricle					
							A	D	A	D	A	D	A	D	A	D	A	D		
							A	D	A	D	A	D	A	D	A	D	A	D		
1	10	2.63	0	251	(7) +63 (3) —24	7 3	8.9	12.2	3.4	3.4	2.44	2.76	1.61	2.23	1.8	1.6	8	2		
2	11	2.4	0.75	207.3	+82.0	6 5	8.16	11.03	3.83	3.48	2.35	2.56	1.75	1.65	1.66	1.70	10	1		
3 A	3	2.72	0.89	242	+40.8	2 1	8.82	6.02	3.35	2.9	2.6	2.5	1.8	1.8	1.7	1.8	3	..		
3 B	4	3.2	0.15	224	+36.6	4 0	10.1	0	2.8	0	2.8	0	1.9	0	2.0	0	3	1		
4	6	2.16	+0.48	200	0	3 4	8.93	8.84	3.13	2.85	2.46	2.63	1.43	1.83	1.6	1.75	0	6		

this reason the weights and measurements of the hearts were divided within the groups. Accordingly, the average weight of the hearts of the rabbits that were killed was the lowest in group 2 (8.16 Gm.), while it was greatest in group 3 B (10.1 Gm.). In proportion to the final body weight, the opposite was true, the hearts of group 2 being  $\frac{1}{203}$  of the average body weight as compared with  $\frac{1}{301}$  of the average body weight in group 3 B. When one considers the ratio of the average final heart weights to the average final body weights, the largest hearts occurred in the rabbits receiving desiccated thyroid and in those animals operated on in addition to being fed the desiccated thyroid, namely, the rabbits in groups 2 and 3 A, respectively. The average weights of the hearts in group 1 are similar to those in the normal control group. In like manner, the hearts in these two groups (2 and 3 A) were the longest (3.83 and 3.35 cm.). Their transverse diameters were also the narrowest but more uniform considering the measurements from the auriculo-ventricular grooves to the apexes. Their left ventricular diameters were, however, comparable to those of the controls (group 4). When the hearts of the rabbits in group 1 are compared with those of groups 2 and 3 A, they are found to be somewhat heavier, shorter and wider at the bases although their apexes were slightly narrower. The left ventricular measurements were similar. Although these weights and measurements of the heart were undoubtedly subject to inaccuracies owing to the manner of death, the retention of blood and the limitations of precision, both human and mechanical, there is evidence to support further the conclusion that the augmented heart rate induced hypertrophy after an interval of time and that there eventually resulted disturbance within its architecture. The latter condition may be due to a disproportion between the heart's load of work and its available nutrition.

#### COMMENT

A comparison of the results shows that lesions were produced in varying degree in the hearts of 80 per cent of the rabbits (group 1) operated on to destroy the inhibitory nerve mechanism, while approximately 90 per cent of the hearts of the rabbits (group 2) made toxic with desiccated thyroid showed similar lesions. It is also evident that a combination of both of these experimental conditions produced more striking results.

The facts that have evolved in this study appear to us to substantiate the claims of other investigators (Goodpasture,<sup>3</sup> Cameron,<sup>4</sup> Hashimoto,<sup>5</sup> Takane<sup>6</sup>) as well as our own that certain lesions do occur in the hearts

3. Goodpasture, E. W.: *J. A. M. A.* **76**:1545, 1921.

4. Cameron, A. T., and Carmichael, J.: *Am. J. Physiol.* **58**:1, 1921.

5. Hashimoto, H.: *Endocrinology* **5**:579, 1921.

6. Takane, K.: *Virchows Arch. f. path. Anat.* **259**:737, 1925.



of rabbits made toxic by the administration of thyroid products (thyroxine). It also seems to us that such lesions are similar to those described in human hearts by Fahr and Kuhle<sup>7</sup> and Goodpasture,<sup>8</sup> although one might question the conclusion of these authors on the basis of the material used for study. Weller's<sup>8</sup> investigation, in which he was unable to find any lesions in the hearts of thirty-five patients with exophthalmic goiter that could not be matched in the hearts of a control series, is at variance with the studies of these authors. We have not had the opportunity to study adequate and reliable human material, since it appears to us that the evaluation of the effects of hyperthyroidism on the human hearts should be made on the basis of the study of the hearts of persons dying of fulminating hyperthyroidism in the earlier decades of life when the cumulative damages of previous cardiac insults may be excluded or minimized. It is our belief that in older persons with oncoming arteriosclerosis, occupational vicissitudes, etc., the cardiac damage induced by an excess secretion of thyroxine cannot be readily separated from other nonspecific traumatism of the cardiac muscles.

All clinical observations point to a basic damage of the myocardium in instances of toxic hyperthyroidism although the disease entity is considered as having a more widespread character (Rake and McEachern<sup>9</sup>). Our studies support this point of view.

If one accepts hyperthyroidism as a disease entity in which certain cardiovascular irregularities become dominant clinically and experimentally, there arises the question of the specificity of a toxic influence of an excess thyroxine content of the blood on the cardiac muscle. Yater<sup>10</sup> expressed the belief that it is "increased thyroxin content in the heart muscle which causes the heart to beat more rapidly and more vigorously" and that there is "associated with this a general vascular relaxation brought about by local action of metabolites on the arterioles and capillaries." Rake and McEachern<sup>9</sup> concluded that hyperthyroidism by itself produces no specific lesions in the myocardium. These authors pointed to the absence of glycogen in the experimentally produced disease, as determined by Hoet<sup>11</sup> and de Fauw,<sup>12</sup> as indicating susceptible undernourished muscle that yields readily to damaging influence. Lehrman and Means,<sup>13</sup> in an analysis of one hundred and eighty-four patients

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7. Fahr, T., and Kuhle, J.: *Virchows Arch. f. path. Anat.* **233**:286, 1921.

8. Weller, C. V.; Wanstrom, R. C.; Gordon, H., and Bugher, J. C.: *Am. Heart J.* **8**:8, 1932.

9. Rake, G., and McEachern, D.: *Am. Heart J.* **8**:19, 1932.

10. Yater, W. M.: *Am. Heart J.* **8**:1, 1932.

11. Hoet, J. P., and Marks, H. P.: *Proc. Roy. Soc., s. B*, **100**:72, 1926.

12. de Fauw, J.: *Compt. rend. Soc. de biol.* **105**:228, 1930.

13. Lehrman, J., and Means, J. H.: *Am. Heart J.* **8**:55, 1932.

with hyperthyroidism, concluded that "it is indicated that hyperthyroidism per se does not produce so-called thyroid heart disease but causes functional derangement in a cardiovascular system already damaged by other pathological conditions." From these citations in the literature, it may be seen that the trend of thought of the investigators is first to question the possibility of segregating specific lesions of the heart in hyperthyroidism in clinical or experimental studies and secondly to point out that if such disturbances do occur they are probably non-specific, resulting from altered conditions in the circulatory system in particular and the body in general.

As stated previously, our own studies lead us to believe that specific lesions can be produced in the hearts of rabbits fed desiccated thyroid within the limits of anticipated individual variations in animals. With regard to the specificity of such lesions, we can only point to the results of our studies in which we attempted to unleash the heart by interference with its stabilizing mechanism. In the rabbits of this series (group 1), it must be remembered, the functional excitation was quite well limited to the heart, there being little or no modification of body weight. It therefore became necessary for such unbalanced hearts to maintain the circulation in otherwise normal rabbits. Under such conditions of rest in cages and with probable individual, variable, cardiac stability and compensation, lesions similar to those occurring in hyperthyroidism appeared in a comparable manner. Although we were not able adequately to demonstrate it microscopically, we strongly suspect that in this group of animals the altered functional strain, the disappearance of glycogen or other nutritional disturbance were responsible for the thyroid heartlike lesions in the rabbits with surgically augmented heart rates.

#### CONCLUSIONS

Comparable myocardial lesions consisting of swelling of the muscle bundles, loss of cross-striation, fat vacuole, histiocytic invasion and fibrosis with fraying of the muscle bundles were produced in varying degree in 80 per cent of the rabbits in which the depressor nerve mechanism to the heart was destroyed by operation and in 90 per cent of the rabbits with toxicity induced by the administration of desiccated thyroid.

Rabbits operated on to destroy the depressor nerve mechanism to the heart and also given desiccated thyroxine to the point of toxicity developed more striking myocardial lesions.

The administration of doses of ephedrine to raise the pulse pressure did not augment the results, but rather lessened them.

These studies support the view that the myocardial lesions found in hyperthyroidism are nonspecific and probably of mechanical and nutritional origin.

# EXPERIMENTAL INTIMAL SCLEROSIS OF THE CORONARY ARTERIES OF RATS

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This paper constitutes a report of some experimental work in which widespread and severe intimal lesions of the coronary arteries were produced in rats by the administration of enormous doses of an agent *which exerts a profound effect on the calcium metabolism.*

## MATERIAL AND METHODS

Fourteen rats, 28 days old, of an average weight of 44 Gm., were used in the experiment. Seven of these were kept in a separate cage and were given, in addition to their regular diet, from 1 to 2 drops per day of activated ergosterol, 10,000  $\times$ . This was given by a dropper by mouth. These rats as well as the seven controls were on our stock laboratory diet. Three of the experimental animals died in the second week of the experiment and were mutilated by the remaining rats so that sections could not be prepared from anything except the bones. One rat died at twenty-one days, and the remaining three were killed on the twenty-second day. The experimental animals lost a slight amount of weight during the experiment, whereas the controls more than doubled their weight.

Autopsy was performed on the three rats which survived for twenty-two days and the one rat which died on the twenty-first day, and tissues were obtained from the heart, aorta, lung, liver, kidney, thyroid gland, parathyroid glands and bones. These were fixed in formaldehyde and sectioned by the paraffin method. Serial sections of the cardiac muscle were cut in each case. They were stained with hematoxylin and eosin.

## OBSERVATIONS

*Coronary Vessels.*—The branches of the coronary arteries in the cardiac muscle showed numerous and severe lesions. They may be interpreted more easily if one is familiar with the lesion produced with one single massive dose of activated ergosterol, 10,000  $\times$ . This has been described in detail (Ham<sup>1</sup>), and the essential finding in it is the tremendous calcification of the media. Cellular structure is often not apparent in this layer, and only a few endothelial lining cells may be seen persisting in the intima.

The medial lesions in the present work are quite obvious, and the dense ring of calcification serves to mark the position of the original

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From the Department of Anatomy, University of Toronto.

1. Ham, A. W.: Arch. Path. **14**:613, 1932.

media. This ring of calcification may be advantageously compared to that resulting from a single upset of calcium metabolism (fig. 4 of previous contribution<sup>1</sup>). In contrast to the lesion produced by a single dose of ergosterol, there may be seen in these experimental animals, in the coronary arteries, a marked proliferation of tissue inside the media. This newly formed tissue encroached on the lumen in varying degrees. The new lumen which had formed in each vessel was lined with very cellular endothelium, which gave the impression of recent proliferation. The main bulk of the newly formed tissue was continuous

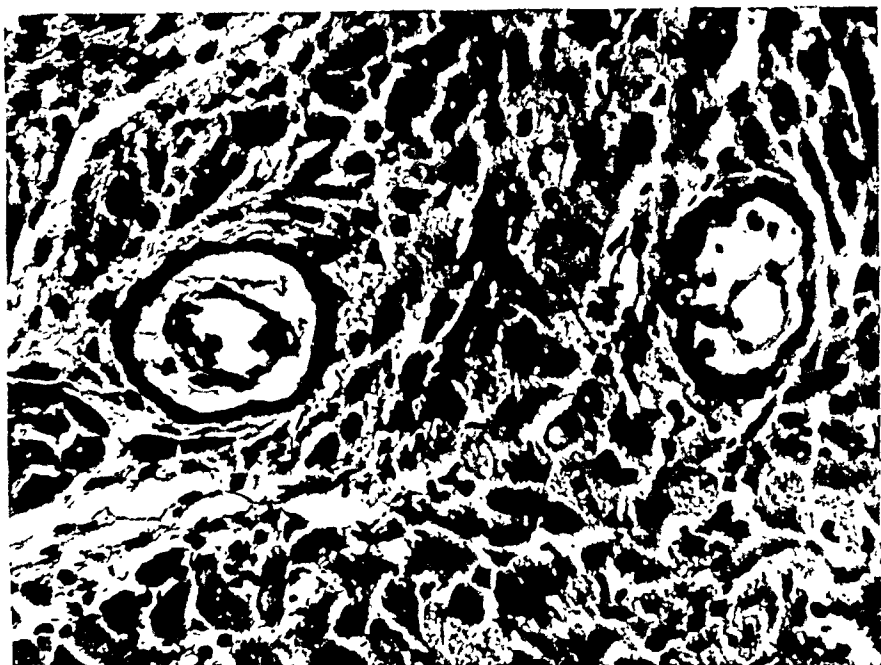


Fig. 1.—Photomicrograph of two coronary vessels showing loosely arranged intimal proliferation. The media of the original vessel wall shows as a heavily calcified ring. Hematoxylin and eosin;  $\times 200$ .

with, and lay directly beneath, the endothelium. In some instances it consisted of loosely arranged vacuolated connective tissue which possessed an embryonic appearance (figs. 1 and 2). In other instances the tissue was young and cellular, but the matrix was more solid. This type of lesion may be seen in figures 3, 4 and 5. This newly formed connective tissue encroached on the lumen in different degrees (figs. 3, 4 and 5). In many instances it led to an almost complete obliteration of the lumen of the vessel. Some of these vessels in which the lumen had been almost completely obliterated gave the appearance usually associated with recanalization.

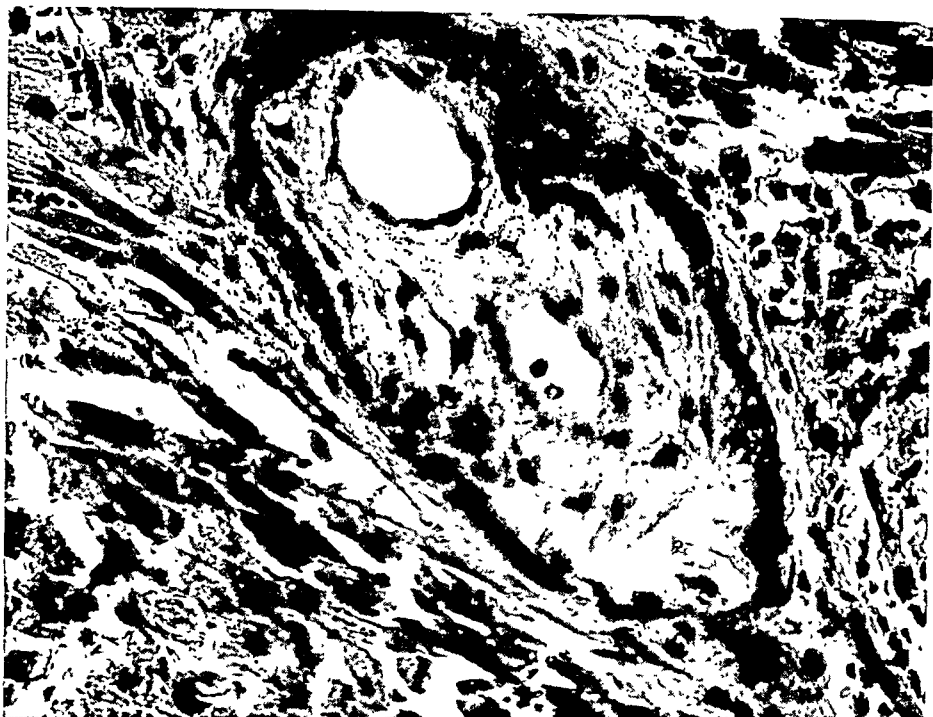


Fig. 2.—Photomicrograph of a coronary vessel showing loosely arranged connective tissue resulting from intimal proliferation. The media of the original vessel wall shows as a heavily calcified ring. The cellular proliferation has left two lumens. Hematoxylin and eosin;  $\times 400$ .

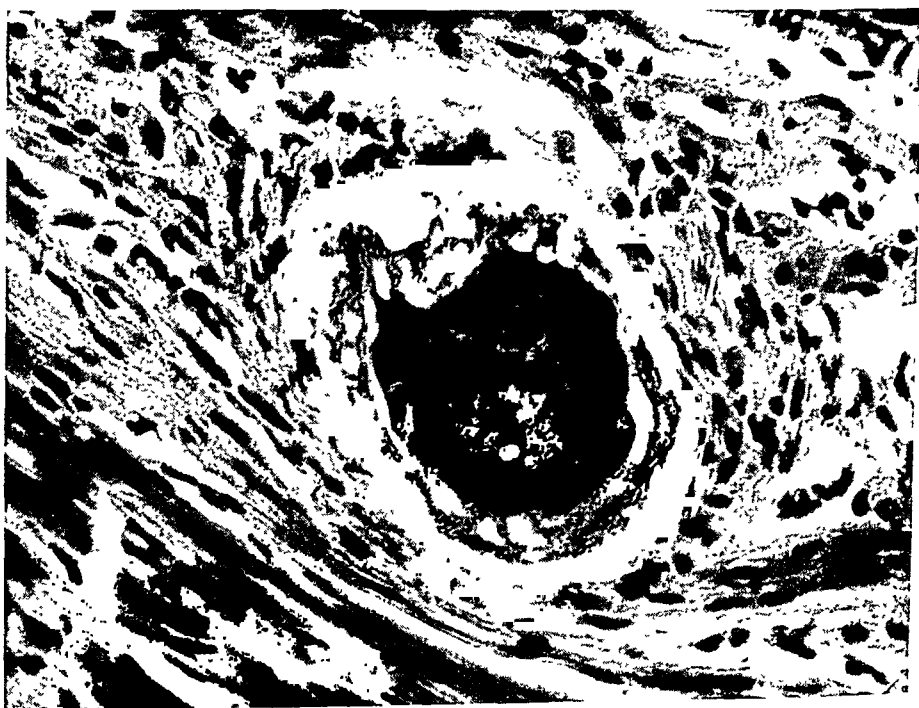


Fig. 3.—Photomicrograph of a coronary vessel showing intimal proliferation of a denser type of tissue. The media of the original wall shows as a heavily calcified ring. Hematoxylin and eosin;  $\times 400$ .

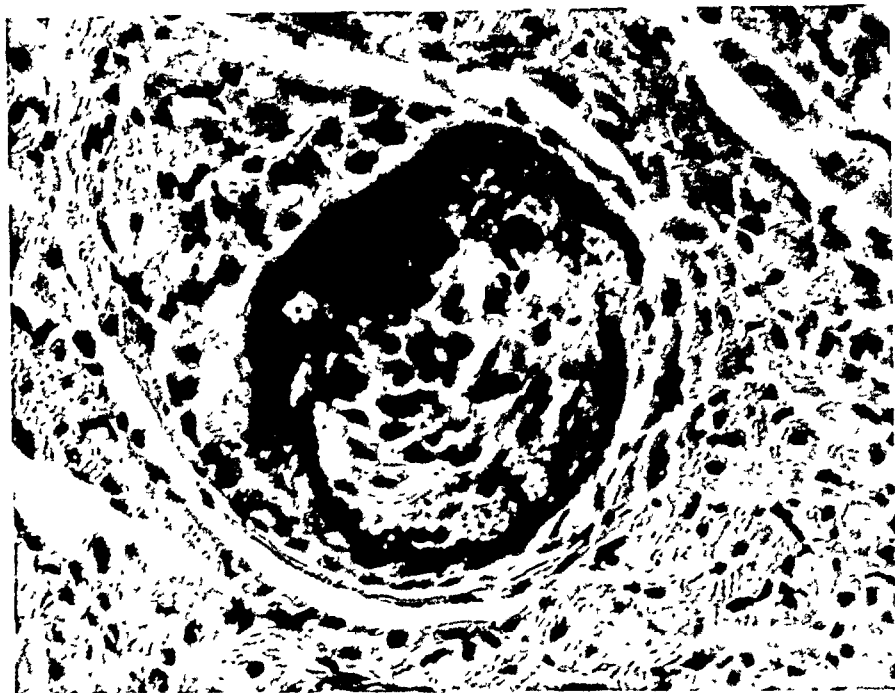


Fig. 4.—Photomicrograph of a coronary vessel in which intimal proliferation has almost occluded the vessel. The media of the original wall appears as a heavily calcified ring. Hematoxylin and eosin;  $\times 400$ .

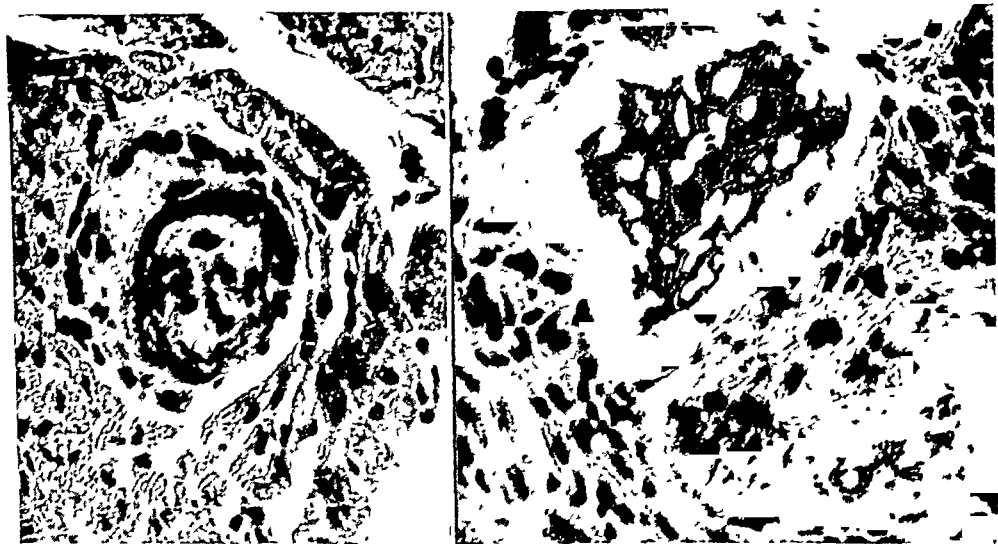


Fig. 5.—Photomicrographs of coronary vessels showing extensive intimal proliferations. The media of the original vessel walls may be seen as heavily calcified rings. Hematoxylin and eosin; left,  $\times 300$ ; right,  $\times 400$ .

*Cardiac Muscle.*—Areas of atrophy of the muscle fibers were found in the myocardium, and these were associated with a real or apparent increase of connective tissue and some hemorrhage.

#### COMMENT

The most important point to discuss in connection with these lesions is their etiology. It is probably misleading to think of them in association with vitamin D because it is improbable that they are produced by the direct effect of this substance on arteries. There is a great deal of evidence to indicate that the medial lesion is produced by a disturbance of mineral metabolism. Vitamin D in special, potent preparations is a convenient agent to use in enormous dosages to cause these disturbances of mineral metabolism. This evidence which points to hypervitaminosis D arteriosclerosis being caused by precipitations of calcium from the serum rather than by preliminary degenerative changes in the arterial tissues may be briefly summarized:

1. The toxicity of vitamin D varies with the amounts of calcium in the animals' diet (Bills and Wirick,<sup>2</sup> Harris and Innes<sup>3</sup>).

2. The same general type of calcification (metastatic) may be produced by overdosages of parathyroid hormone.

3. The medial calcifications occur suddenly following a latent period after the administration of a single massive dose of vitamin D (Ham<sup>1</sup>).

4. The preceding changes in the arterial walls are by no means sufficient to account for the tremendous calcification which occurs a few hours later (Ham<sup>1</sup>).

5. The time of calcification of the vessel wall corresponds with the beginning of the fall of the level of serum calcium after the attainment of a hypercalcemia when one dose of vitamin D is used (Ham and Portuondo<sup>4</sup>).

If the cause of the medial lesion seen after the administration of a single massive dose of activated ergosterol is considered to be the precipitation of calcium from the blood, it becomes necessary to visualize a different sort of sequence of events in this type of arteriosclerosis from that which has been postulated to explain the development of the usual lesion. In hypervitaminosis D arteriosclerosis the deposition of calcium is an early event rather than a late one, and instead of injury being followed by degeneration or proliferation, then calcification, the order is reversed and calcification is followed by degeneration and proliferation.

The intimal proliferation could be explained in several different ways. It might be suggested that it is in the nature of a hyperplasia to compensate for an injured media. It is conceivable that repair might

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2. Bills, C. W., and Wirick, A. M.: *J. Biol. Chem.* **86**:117, 1930.

3. Harris, L. J., and Innes, J. R. M.: *Biochem. J.* **25**:367, 1931.

4. Ham, A. W., and Portuondo, B. C.: *Arch. Path.* **16**:1, 1933.

ensue from the intima when the media is so severely damaged by precipitation of calcium. The regeneration of tissue from the intima is, however, sufficiently extensive to suggest that it has been stimulated to grow by more than one shower of calcium. Whereas the effect of the first precipitation seems to be primarily on the media of vessels, it is not improbable that after the media has become well calcified subsequent precipitations would injure the intima to a greater extent than before. Granules of calcified material could be seen in the cellular masses resulting from the intimal proliferation, particularly in the portion of these close to the media.

Whereas it seems probable that precipitations into vessel walls occurred on several occasions during the course of the experiment, it is possible that the deposit of calcium in the wall of the media could also act as an irritant in its immediate neighborhood. There is some evidence to show that calcium may gradually disappear from arteries (Spies<sup>5</sup>), and this problem is now being investigated in this laboratory. It is possible that the redissolving of the primary deposit might be accompanied by some local disturbances which would stimulate cell growth.

The significance of this work does not appear to us to lie in the fact that the lesions may be caused by hypervitaminosis D. It would appear to us that these intimal lesions, as well as the medial lesions, are the result of nothing more than the inability of the blood to retain, on certain occasions, all of its calcium in solution. The possibility of disturbances of calcium metabolism being a factor in the etiology of human arteriosclerosis should be considered more seriously than it is at present. The blood normally contains more calcium than can be explained by the laws of simple solution. It is possible that there are many ways in which small amounts of calcium could be precipitated from the blood.<sup>6</sup> Whether this mechanism accounts for any lesions in man remains to be seen, but the ease with which endarteritis in the coronary vessels may be produced by this particular method of disturbing calcium metabolism suggests that this etiology of arterial disease (precipitation of calcium) should at least be kept in mind.

#### CONCLUSIONS

Proliferative changes of a marked character in the intima of the coronary arteries of rats occurred following the daily administration of enormous dosages of activated ergosterol, which profoundly affects the calcium metabolism. It is our opinion that whereas the first effect of the precipitation of calcium from the serum is the calcification of the media, further precipitations or the later effects of the first precipitation result in intimal proliferation.

5. Spies, T. D.: *Arch. Int. Med.* **50**:443, 1932.

6. Ham.<sup>1</sup> Ham and Portuondo.<sup>4</sup>



# HEPATIC AND BILE DUCT CHANGES FROM OBSTRUCTION OF COMMON BILE DUCT DUE TO PANCREATIC CARCINOMA

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Early writers suspected in a general way that long-continued obstruction to the common bile duct might result in increased fibrosis within the liver, although comparatively little attention was paid to this condition in human beings. Albers<sup>1</sup> described the liver in such instances as granular, rough and yellowish brown, with atrophy and induration of the parenchyma, and the probable pathogenesis of these changes was discussed by Virchow.<sup>2</sup> Förster,<sup>3</sup> Cohn<sup>3</sup> and Legg<sup>4</sup> distinguished, as a special type, the cirrhosis arising in connection with biliary obstruction. Charcot and Gombault<sup>5</sup> described similar hepatic lesions resulting from calculi in the ampulla of Vater and in the gallbladder, cancer of the head of the pancreas and angiocholitis of the larger bile ducts. Mangelsdorff,<sup>6</sup> Ruppert,<sup>7</sup> Janowski,<sup>8</sup> Sauerherring<sup>9</sup> and Ford<sup>10</sup> individually emphasized particularly the changes in the bile ducts, the proliferation of the connective tissue or the occurrence of multiple necroses in the

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From the Pathological Laboratories of the Jefferson Medical College and Hospital, the Jefferson Hospital Tumor Clinic and the Philadelphia General Hospital.

1. Albers, J. F. H.: *Beobachtungen auf dem Gebiete der Pathologie und pathologischen Anatomie*, Bonn, König und van Borcharen, 1836, p. 19.

2. Virchow, R.: *Verhandl. d. phys.-med. Gesellsch.* **7**:21, 1857.

3. Förster, cited by Cameron, G. R., and Oakley, C. L.: *J. Path. & Bact.* **35**:769, 1932.

4. Legg, J. W.: *St. Barth. Hosp. Rep.* **9**:161, 1873; *Tr. Path. Soc. London* **25**:132, 1874; *On the Bile, Jaundice and Bilious Diseases*, London, H. K. Lewis & Co., Ltd., 1880.

5. Charcot, J. M., and Gombault, A.: *Arch. de physiol. norm. et path.* **8**:272, 1876.

6. Mangelsdorff, J.: *Deutsches Arch. f. klin. Med.* **31**:522, 1882.

7. Ruppert, H.: *Zur Frage über die Entstehung der biliären Lebercirrhose*, *Pam. Towarz. lek. Warszaw* **87**:324 and 531; **88**:531, 1891.

8. Janowski, W.: *Beitr. z. path. Anat. u. z. allg. Path.* **11**:344, 1892.

9. Sauerherring, H.: *Virchows Arch. f. path. Anat.* **137**:155, 1894.

10. Ford, W. W.: *Am. J. M. Sc.* **121**:60, 1904.

liver affected by stasis. Eppinger<sup>11</sup> reported a case which may be regarded as classic and in which the common bile duct was inadvertently severed during an operation and immediately ligated 2 cm. distal to the junction of the hepatic and cystic ducts. Icterus and bilirubinemia appeared within thirty-six hours, and death from aspiration pneumonia occurred at the end of forty-eight hours. At autopsy, the question of infection of the biliary tract and of circulatory disturbances was ruled out; microscopically, there were pigmentary and regressive changes, especially in the central cells of the lobules, and dilatation and elongation of the bile capillaries, with rupture of their distal ends into the perivascular lymph spaces. Studies of biliary stasis in man have been contributed by Weber,<sup>12</sup> Abramow and Samoilowicz,<sup>13</sup> Carnot and Harvier,<sup>14</sup> Ogata,<sup>15</sup> Zypkin<sup>16</sup> and Rabl.<sup>17</sup>

Heineke<sup>18</sup> was one of the first to distinguish two distinct types of obstructive biliary cirrhosis in man, the one complicated by infection and the other uncomplicated. More recently Fiessinger, Albot and Dieryck<sup>19</sup> referred to the hepatic lesions resulting from pure mechanical stasis as cholestatic cirrhosis in contrast to the infectious type or cholangitic cirrhosis. MacMahon and Mallory<sup>20</sup> described the characteristics of noninfectious obstructive cirrhosis; their findings, however, are open to the possible criticism that infection may have been a complicating factor in the cases in which there were calculi and acquired strictures of the common duct and perhaps also in some of those in which carcinoma of the ampulla of Vater was present.

In animals, aseptic ligation of the common bile duct is a simple procedure, as a result of which there is a vast literature on the subject, but one of the few diseases in man which appropriately reduplicates this experimental lesion and adequately fulfils the requirements of the condition of uncomplicated biliary stasis is obstruction to the common bile duct by carcinoma of the head of the pancreas. This condition is comparatively uncommon, and before death there is usually only a relatively short period of stasis, the effects of which are further mitigated to a

\* 11. Eppinger, H.: *Beitr. z. path. Anat. u. z. allg. Path.* **31**:230, 1902.

12. Weber, F. P.: *Tr. Path. Soc. London* **54**:103, 1903.

13. Abramow, S., and Samoilowicz, A.: *Virchows Arch. f. path. Anat.* **176**:199, 1904.

14. Carnot, P., and Harvier, P.: *Arch. de méd. expér. et d'anat. path.* **19**:76, 1907.

\* 15. Ogata, T.: *Beitr. z. path. Anat. u. z. allg. Path.* **55**:236, 1913.

16. Zypkin, S. M.: *Virchows Arch. f. path. Anat.* **262**:791, 1926.

\* 17. Rabl, R.: *Beitr. z. path. Anat. u. z. allg. Path.* **86**:135, 1931.

18. Heineke, H.: *Beitr. z. path. Anat. u. z. allg. Path.* **22**:259, 1897.

19. Fiessinger, N.; Albot, G., and Dieryck, J.: *Ann. d'anat. path.* **8**:537, 1931.

\* 20. MacMahon, H. E., and Mallory, F. B.: *Am. J. Path.* **5**:645, 1929.

variable extent by the functional activity of the gallbladder, which is usually normal. Judd, Counseller and McIndoe<sup>21</sup> have emphasized the development of hydrohepatosis in association with obstruction to the common bile duct by pancreatic carcinoma, in which condition they felt that, owing, among other things, to the absence of infection, there is a minimum of obstructive biliary cirrhosis. Rolleston<sup>22</sup> has maintained that in man cirrhosis does not result from uncomplicated biliary obstruction per se. The paucity of knowledge of the hepatic lesions produced in the condition of uncomplicated biliary stasis was recently emphasized by Rössle,<sup>23</sup> who pertinently stated: "Besieht man kritisch die Literatur, so schwindet die ungeheure Masse der angeblich hierher gehörigen Beobachtungen auf ein Häufchen zusammen." (If one reviews the literature critically, the enormous mass of observations that apparently belong in this group shrinks to minute proportions.)

The purpose of the present communication is to describe the changes in the hepatic and bile ducts resulting from obstruction to the common bile duct by carcinoma of the head of the pancreas, and to submit evidence showing that cirrhosis may result from uncomplicated or non-infectious biliary stasis. The material for this study was obtained from twenty-four cases of primary carcinoma of the head of the pancreas with obstruction to the common bile duct. Seven of these cases are from the pathologic laboratory of the Pennsylvania Hospital; Dr. John T. Bauer placed this material at our disposal. None of the cases was complicated by previous cholecystitis or cholelithiasis, terminal abscesses or operative decompression of the biliary system, and in all of them comprehensive records and suitable gross or histologic material or both were available for use. Histologic material was fixed in formaldehyde or Zenker's fluid; some of it was frozen and sectioned, and the remainder was blocked in paraffin, cut and stained with phosphotungstic acid, hematoxylin-eosin, scarlet red, methylthionine chloride, U. S. P. (methylene blue), van Gieson's and Mallory's connective tissue stains, Verhoeff's elastic tissue stain, oxidase stain, iron-alum-hematoxylin stain, Wilder's modification of the silver diaminohydroxide stain, Mallory's potassium ferrocyanide stain and McIndoe's adaptation of the Hortega silver carbonate stain for bile canaliculi. In many instances complete sets of serial sections were also examined.

21. (a) Judd, E. S., and Counseller, V. S.: *J. A. M. A.* **89**:1751, 1927. (b) Judd, E. S., and McIndoe, A. H.: *Collected Papers of the Mayo Clinic and Mayo Foundation*, Philadelphia, W. B. Saunders Company, 1929, vol. 21, p. 158. (c) Counseller, V. S.: *Ann. Surg.* **87**:210, 1928.

22. Rolleston, H.: *Diseases of the Gallbladder and Bile-Ducts*, in Christian, H. A., and Mackenzie, James: *Oxford Medicine*, New York, Oxford University Press, 1932, vol. 3, pp. 390 and 469.

23. Rössle, R., cited by de Josselin de Jong, R.: *Compt. rend. de conf. internat. de path. geogr.*, 1931, p. 38.

Metastatic nodules were present in the liver in many of the cases included in this study. The changes in the hepatic tissue adjacent to metastases are well known, and since they have no bearing on the changes under consideration, they will not be discussed further.

#### GROSS CHANGES

The common bile duct averaged 3 cm. in diameter, the dilatation usually being uniform, although sacculations occasionally occurred; the right hepatic duct was less distended than the left, and the intrahepatic ducts suffered least. The walls of the larger biliary passages first become attenuated and subsequently undergo slight thickening. In the early stages the contents of the bile passages are not much altered but later they become thick and tenacious and contain precipitated granules of inspissated pigment. In two cases, the contents of the gallbladder and ducts consisted of thin, pale, yellowish-green fluid. The liver showed a mixture of yellow, olive-drab and deep green mottling, and was usually enlarged and frequently smooth, although in four cases it was smaller than normal and in nine cases the surface was either finely or coarsely granular. Weights of from 1,800 to 2,000 Gm. were recorded in cases in which metastatic nodules were few or absent. In two cases the liver weighed 810 Gm. and 840 Gm., respectively. Varicose ducts may appear on the surface, and the organ may feel cystic. In the cases lasting a long time, the liver was firm, tough and heavy, cut with increased resistance and on section disclosed enormously dilated biliary passages and visible small islands of connective tissue which in a few instances was a well marked feature. The intrahepatic bile passages were usually filled with deep green or greenish-black, thick, viscid bile, which in two cases was pale yellow. The lobular markings were accentuated by contrast with the darkly colored centers and light peripheries.

#### MICROSCOPIC EXAMINATION

Bile pigmentation is a variable feature although its intensity is always greatest about the central vein and branches of the hepatic vein. Within hepatic cell cytoplasm bile pigment occurs in three forms: (1) as a diffuse yellowish-green staining substance without precipitation, (2) as fine, golden brown granules and (3) as variously sized, yellowish-green or greenish-black droplets. It may be scattered diffusely throughout the cell, or may be clumped as small foci at one or both poles of the nucleus, which it may completely encircle or occasionally overlie and obscure. Rarely, the nucleus itself seems to have taken up some of the pigment. In the intracellular, cytoplasmic vacuoles, the outer circumference may show a concentration of the pigment, which may also be contained as fine granules within the vacuole itself. Occasionally a

narrow zone of the hepatic cell cytoplasm bordering the canaliculus is deeply pigmented. Bile thrombi or bile cylinders are constantly present, and may be rod-shaped or cylindric, occasionally branched and sometimes markedly irregular owing to their projection into collateral canaliculi. In the necrotic areas bile thrombi are discharged from the canaliculi into the tissue spaces and in the later stages into the sinusoids; in both situations they may be found lying free or phagocytosed in part or in whole. Bile masses also form casts in a number of the smaller interlobular ducts which on rupture permit extrusion of their contents into the adjacent stroma or into a dilated lymphatic. Monocytes laden with bile pigment are found within the lobule, in the stroma of Glisson's capsule and wherever else a reactive or reparative process is in progress. In the peripheral and central portions of the lobules of certain of the livers iron pigment was present in the hepatic and Kupffer cells, and what appeared to be lipochromes were present in the hepatic cells.

About the central vein and branches of the hepatic vein, the hepatic cells show regressive changes which vary in intensity from simple parenchymatous degeneration to actual necrosis and ultimate dissolution. Approaching the periphery of the lobule, the cells retain more of their normal appearance and regular cordlike arrangement, although less marked sporadic regressive changes may be present. Occasionally a narrow band showing pigmentation and regressive changes may radiate from the central vein to the portal radicle, where it is not uncommonly joined by a similar band from an adjacent lobule. Atrophy of the hepatic cells is noted in association with dilated sinusoids and is present especially at the periphery of the lobules, where irregularly distributed intracellular cytoplasmic vacuoles may be found.

Uniform distention of bile capillaries is a constant early feature, and, with subsequent disintegration of the polygonal cells, on the integrity of which the continuity of the canalicular system depends, rupture takes place. The dilating collateral canaliculi not infrequently extend to the perivascular tissue space, exerting pressure against and indenting the reticular wall, which finally gives way at this point with resulting extrusion of the canalicular contents into the sinusoid. Pictures suggesting the existence of intracellular canaliculi are not observed. There is no blood present within canalicular lumens.

The arteries and arterioles show an increase in the medial musculature and less frequently subintimal proliferation which may result in partial or complete obliteration of the lumens. The branches of the portal vein are regularly dilated, and their walls are thickened by a perivascular fibrosis; their lumens are occasionally occluded by mottled or by tumor thrombi. The lymphatic vessels in Glisson's capsule are dilated and at times contain bile pigment. The central vein is usually dilated, and its wall is variably thickened by hyalinized connective tissue.

Branches of the hepatic vein are dilated and congested and may contain a few lymphocytes and single or multinucleated macrophages laden with bile pigment; their walls are considerably thickened by hyalinized connective tissue. Sinusoidal hyperemia is encountered regularly about necrotic areas, occurs indifferently in areas of atrophy of the hepatic cells and is a variable feature, depending in many instances on extraneous factors. In cases of long duration there is an appreciable increase of reticulum in the walls of the sinusoids, the lumens of which may contain swollen, pigmented or nonpigmented Kupffer cells and monocytes, an occasional lymphocyte and polymorphonuclear leukocyte, iron pigment and free bile pigment in the form of droplets, coarse granules and bile masses. Rupture of the sinusoidal walls, permitting a direct communication with tissue spaces and with bile canaliculi, is a late feature.

In areas where bile pigmentation is marked or regressive changes are in progress, the Kupffer cells are prominent and contain bile pigment, nuclear fragments and other débris. In necrotic areas these cells also show regressive changes but remain remarkably well preserved long after complete dissolution of the polygonal cells has occurred. When disintegration sets in, the nucleus disappears before the cytoplasm breaks up.

Monocytes are constantly found in the interlobular connective tissue, surrounding and invading areas of necrosis, within sinusoids and other blood vessels and occupying the perivascular tissue spaces. Together with the Kupffer cells, from which they are at times difficult to distinguish, they manifest marked phagocytic properties and occasionally form multinucleated giant cells.

Aside from the regressive changes occurring about the central veins, there were two other distinctive types of necrosis:

1. In sixteen of the cases, small, isolated, rectangular or oval, sharply demarcated, focal necrotic areas, consisting at first of from 8 to 10 polygonal cells, were found in the midzones of many of the lobules. The regressive changes in these areas were characterized by: (*a*) fading and shrinking of the nuclei and fading of the cytoplasm of the hepatic cells with accentuation of the peripheral cellular outline due partly to swelling and concentration of the reticulum, (*b*) a relatively greater resistance of the Kupffer cells toward retrogressive changes, (*c*) a moderate infiltration of monocytes and (*d*) a sharply defined peripheral limit, with normal cells on the outside and necrotic cells within. The amount of pigment contained in these areas was not greater than that present in the adjacent unaffected cells, and gradually diffused out to the periphery where it was engulfed by inwandering phagocytic cells. This lesion may enlarge by projecting its apex to and sometimes beyond the central vein,

and more frequently by extending its broad base into the outer third of the lobule, reaching at times to the portal radicle, where it may be met by a similar lesion (fig. 1). The area was usually bloodless but was not infrequently surrounded by a hyperemic and hemorrhagic zone, into which the necrosis might extend secondarily. A mantle of monocytes and lymphocytes was noted about those recently formed. Within the lesion the normal hepatic architecture was in most instances maintained,

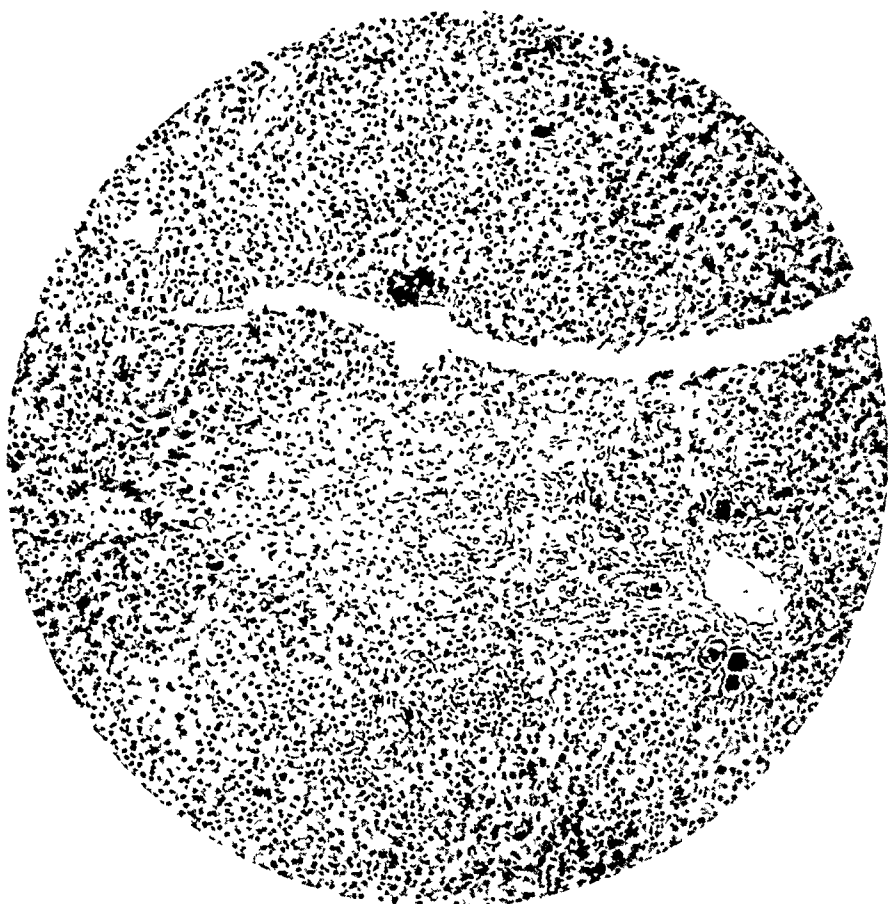


Fig. 1.—An enlarged focal midzonal area of necrosis with its apex at the central vein on the left and its base at the portal radicle on the right. Note also the independent hepatic cell pigmentation about the central vein;  $\times 69$ .

but finally, with complete dissolution of the cells, nothing remained but a loose meshwork of condensed, thickened, hyalinized, reticular fibers with little or no attempt at organization.

2. In five cases large, oval or triangular, sharply demarcated biliary necroses were present. These occupied, as a rule, the outer portions of the lobules, although some were centered in the portal radicles to which they were entirely confined or from which they extended to involve the peripheries of adjacent lobules. When first formed, the center of

this lesion is composed of a dense, compact, sharply circumscribed, dark green mass of bile pigment, occasionally containing a few scattered ductal epithelial cells. This mass, occupying an intralobular situation, becomes surrounded by a narrow concentric zone of degenerated and necrotic hepatic cells which maintain for some time their normal cord-like arrangement, separated by congested sinusoids lined by relatively unchanged Kupffer cells (fig. 2). Beyond this zone the sinusoids are

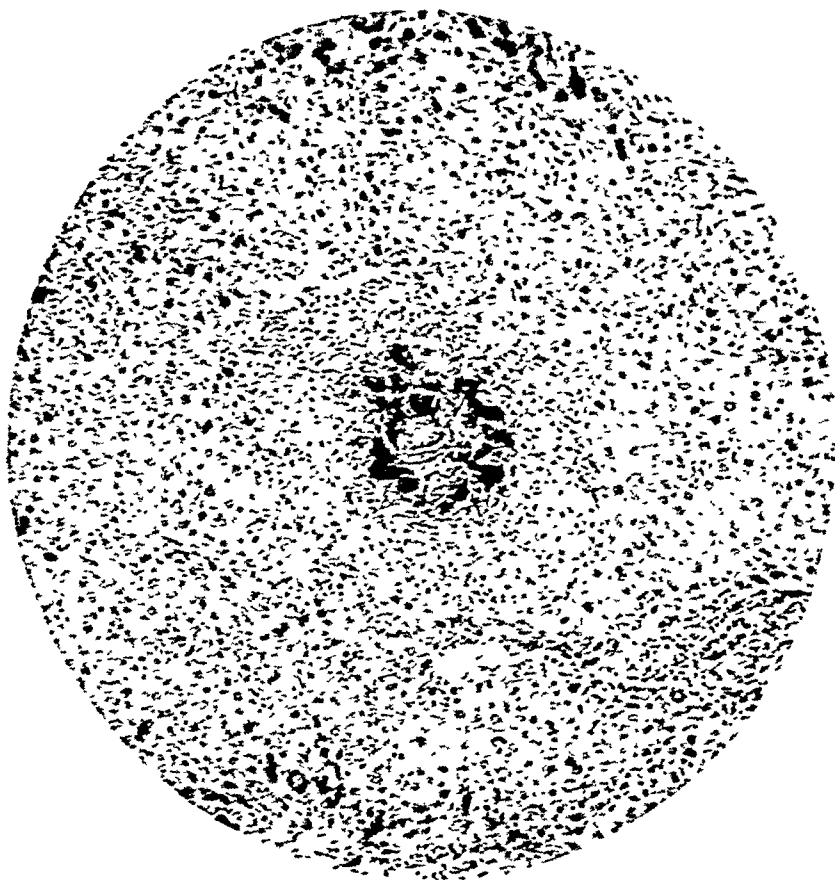


Fig. 2.—Intralobular biliary necrosis is present in the center, with a portal radicle below and to the right. Note the disintegration of the central masses of bile pigment, which are separated by coarse reticular fibers impregnated with bile pigment. The surrounding necrotic zone, which is sharply demarcated from the normal hepatic tissue, contains swollen Kupffer cells and infiltrated polyblasts:  $\times 82$ .

markedly congested and filled with normal or conglutinated erythrocytes. As the lesion progresses the necrotic zone becomes paler and wider, and a fine hepatic cell cytoplasmic vacuolation appears, followed by karyorrhexis and other nuclear changes. The peripheral portion of the central mass of pigment gradually diffuses into the necrotic zones. Coincident with this monocytes wander in and form irregularly distorted, multi-



nucleated giant cells, and the pigment is found in smaller homogeneous and coarsely granular, discrete aggregations. A little later, in the center of the biliary necrosis a swollen, reticular, beaded framework, impregnated diffusely or with coarse granules of bile pigment, becomes apparent, and in the interstices of their fibers many swollen monocytes may be noted. Ultimately, the central mass completely disappears, leaving only a coarse golden yellow reticulum. The lesion becomes more

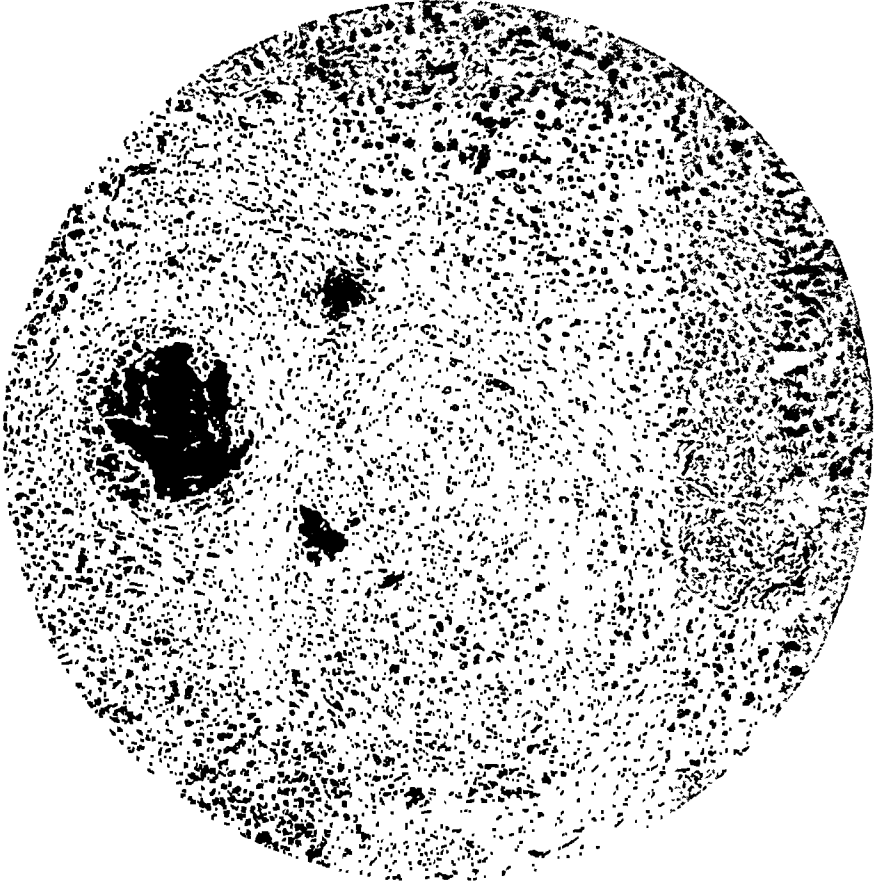


Fig. 3.—Organizing biliary necrosis contributing to perilobular fibrosis. The necrosis is confined to the portal radicle, and shows little diffusion of the central mass of bile pigment;  $\times 103$ .

or less completely surrounded by large swollen monocytes and lymphocytes accompanied by a few fibroblasts, which may proliferate rather rapidly to form a complete, sharply delimiting wall. Finally the entire outer zone is replaced by more or less dense hyalinized connective tissue which, following its usual course of slow contraction, encroaches on the central contents, rendering them more compact. The biliary necroses occurring within the portal areas show little diffusion of the central masses of bile pigment and only slight retrogressive changes in the surrounding connective tissue, which quickly forms a dense capsule about the lesions, which may persist in this form indefinitely (fig. 3).

The bile ducts become elongated, stretched and tortuous, and in addition show a numerical increase, as evidenced by budding and branching and by proliferation of the lining epithelial cells with the formation of syncytial masses (fig. 4). Regressive changes, consisting of pigmentation, degeneration, atrophy, necrosis and desquamation of individual or isolated groups of cells and rarely of denudation en masse of the lining of the duct leaving a bare basement membrane, are incon-

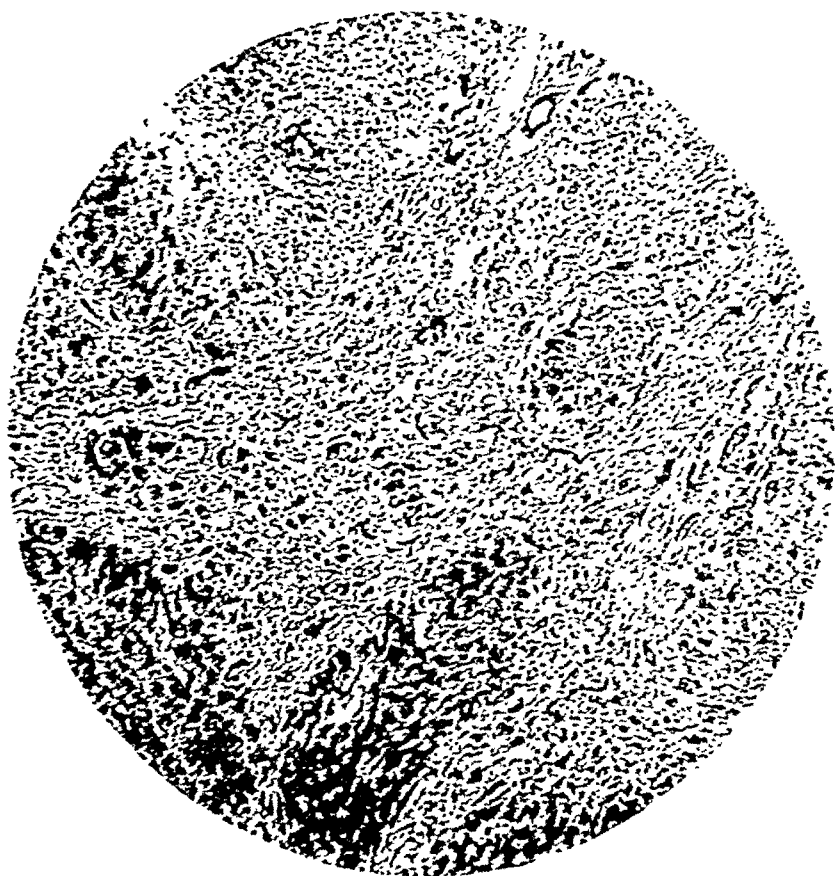


Fig. 4.—Extensive proliferation of the bile ducts and connective tissue;  $\times 60$ .

spicuous features. The lumens may be empty or filled with bile thrombi, or with a pale, homogeneous, pink-staining material. The walls of the larger ducts are thickened, their lumens are dilated, and the epithelial lining cells usually appear fairly normal.

The portal areas, appearing frequently as large oval structures, show an increase in connective tissue which, in the early stages, closely parallels the changes in the bile ducts but which subsequently develops independently, insinuating itself for varying distances between adjacent lobules, where it joins prolongations from contiguous portal areas. These branching processes invade the outer and, at times, the middle third of the lobule. The individual or small groups of hepatic cells thus

enmeshed gradually atrophy and finally tend to undergo complete disintegration. As a result, the lobule becomes irregular in outline and reduced in size. With total stasis existing for more than two months, proliferation of the connective tissue becomes a striking feature, forming regularly an incomplete, and at times a complete, perilobular collar of fibrosis (figs. 3 and 4). Well developed bands of fibrous tissue surrounding the majority of the lobules were present in only one of our cases. In several others the process stopped just short of this. The subcapsular proliferation of connective tissue was marked in the majority of the cases. The organization of the biliary necroses contributed in certain instances to the perilobular increase of connective tissue (fig. 3). Monocytes, lymphocytes and an occasional granulocyte, as well as masses and granules of bile pigment, free and in phagocytic cells, were present within the newly formed connective tissue, which consisted essentially of young fibroblasts with an abundance of coarse, edematous, fibrillar material, directly continuous with the increased reticulum of the periphery of the lobule.

#### COMMENT

Legg,<sup>4</sup> Weber<sup>12</sup> and later McMaster, Broun and Rous<sup>24</sup> emphasized the analogy between hydronephrosis and biliary stasis (hydrohepatosis). In both, as in conditions of obstruction to the ducts of secreting glands in general, secretory stasis, dilatation of ducts, vascular disturbances from collateral pressure and parenchymal atrophy occur. In the case of the liver, however, the activities of the gallbladder partially alleviate the effects of stasis. After total obstruction, the hepatic cells continue to excrete until the pressure in the conducting system rises to a certain point (350 mm. of bile in the dog), when the product of their activity is returned to the organism. Pyroxylin (celloidin) casts of the biliary and vascular channels in the condition of obstruction of the common duct by carcinoma of the head of the pancreas demonstrate a tremendous volumetric increase of these channels (Judd, Counsellor and McIndoe).<sup>21</sup> Judd, Counsellor and McIndoe believed that the intimate relationship between the biliary and vascular passages creates an ideal situation for the production of venous strangulation with consequent diversion of the portal blood current and anemia of the hepatic tissue. The parietal sacculi or diverticuli are effaced, with the result that the walls become smooth. The vasa aberrantia either are flattened out along the walls of the ducts or become enlarged, sacculated and indistinguishable from bile ducts.

In two of our cases, the ductal contents were thin and pale yellow, but "white bile" was not encountered, probably because the obstructed

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24. McMaster, P. D.; Broun, G. O., and Rous, P.: *J. Exper. Med.* **37**:685, 1923.

biliary passages were not the seat of previous disease. In cases of long-standing obstruction, the wall of the gallbladder hypertrophies and undergoes some fibrous thickening, which is probably due, as in the walls of the ducts, to the continuous absorption of bile through the lymphatics. This would tend ultimately to abolish the functions of the gallbladder and predispose to the formation of white bile.

To account for the deeper pigmentation of the center of the hepatic lobule, Legg<sup>4</sup> reasoned that the cells nearest the bile ducts could more easily discharge their contents. Bürker,<sup>25</sup> however, believed that the hepatic lobule consists of a central secreting and a peripheral absorbing portion. As for the bile thrombi, Wyss<sup>26</sup> and Legg regarded them merely as casts of the bile capillaries, but subsequent investigators considered them as an expression of toxic damage to hepatic cells and to be composed of coagulated, inspissated, bile-stained protein (Eppinger,<sup>4</sup> Ogata<sup>15</sup> and Lepehne<sup>27</sup>) or a mixture of lipid and protein (Rous<sup>28</sup>). Kretz<sup>29</sup> believed that the bile thrombi result from destruction of erythrocytes by the action of bile acids with subsequent iron pigmentation of the hepatic cells. Aschoff<sup>30</sup> emphasized Kodama's<sup>31</sup> finding that in obstructive icterus iron liberated from broken-down hemoglobin is retained in the Kupffer cells and appears only in very small quantities in the polygonal cells of the liver. The occurrence of iron pigment in our cases seems to be of no particular significance either as a criterion or as a contributing factor in the production of the hepatic lesions. Hepatic lipochromes, which Connor<sup>32</sup> demonstrated microscopically and by chemical analysis, have been regarded by Ogata as autochthonously associated with the lipoids present in the liver, which, however, contains no neutral fat.

The vacuoles which occur indiscriminately within the hepatic cells have been considered as mucinous (Chambard<sup>33</sup>) or fat droplets (Canalis,<sup>34</sup> Lehoussé<sup>35</sup> and Gerhardt<sup>36</sup>) or as representing a stage

\* 25. Bürker, K.: *Arch. f. d. ges. Physiol.* **83**:241, 1901.

26. Wyss, O.: *Virchows Arch. f. path. Anat.* **35**:533, 1866.

27. Lepehne, G.: *Ergebn. d. inn. Med. u. Kinderh.* **20**:221, 1921.

28. Rous, P.: *Am. J. M. Sc.* **170**:625, 1925.

29. Kretz, R., in von Krehl, L., and Marchand, F.: *Handbuch der allgemeinen Pathologie*, Leipzig, S. Hirzel, 1913, vol. 2, p. 462.

30. Aschoff, L.: *Klin. Wchnschr.* **11**:1620, 1932.

31. Kodama, M.: *Beitr. z. path. Anat. u. z. allg. Path.* **73**:187, 1925.

32. Connor, C. L.: *Am. J. Path.* **4**:235 and 293, 1928.

33. Chambard, E.: *Arch. de physiol. norm. et path.* **4**:718, 1877.

34. Canalis, P.: *Internat. Monatschr. f. Anat. u. Physiol.* **3**:216, 1886.

35. Lehoussé, E.: *Arch. de biol.* **7**:187, 1887.

36. Gerhardt, D.: *Arch. f. exper. Path. u. Pharmacol.* **30**:1, 1892; *Verhandl. d. Cong. f. innere Med.* **15**:460, 1897; *München. med. Wchnschr.* **52**:889, 1905.

of necrosis resulting from circulatory disturbances (Tischner<sup>37</sup>). We agree with Ogata that they are not due to infiltration of fat or glycogen and probably represent a phase of hydropic degeneration.

In his case Eppinger noted dilatation of the bile capillaries and elongation of their distal ends with rupture into the lymph spaces. This finding is most unusual, for in our cases in which obstructive jaundice was present from seventy-two hours onward we were unable to observe ruptures except in those lasting a longer period. A number of investigators (Harley,<sup>38</sup> Frey,<sup>39</sup> Jagic,<sup>40</sup> Abramow,<sup>41</sup> Abramow and Samoilewicz,<sup>42</sup> Tischner<sup>37</sup> and Naunyn<sup>43</sup>) advanced the view that rupture of bile capillaries occurs directly into the blood stream. Liebermeister<sup>43</sup> felt that with increasing pressure in the canaliculi the bile may diffuse, without rupture, directly into the lymph or blood stream, or, as stated by Minkowski,<sup>44</sup> bile pigments may theoretically easily pass by the process of parapedesis from the damaged hepatic cell directly into the blood stream or perilymphatic space instead of into the lumen of the canaliculi. Sterling<sup>45</sup> could not find ruptured canaliculi until comparatively late in the condition, and Ogata<sup>15</sup> was unable to demonstrate any time relationship between the appearance of icterus and visible canicular ruptures. Studying the livers of dogs with obstruction to the common bile duct, Barron and Bumstead<sup>46</sup> suggested that bile merely diffuses from the canaliculi into the perivascular tissue spaces, since only dilatation of the canaliculi with formation of bile thrombi could be demonstrated within from six to eleven hours (Barron and Bumstead,<sup>46</sup> Hiyeda<sup>47</sup> and Kodama<sup>31</sup>), and ruptures were not evident within three days (Bloom<sup>48</sup>). Rabl<sup>17</sup> and Scheunert<sup>49</sup> concluded that in human beings and in dogs obstructive icterus does not depend on rupture of the bile capillaries. Haberland<sup>50</sup> and Garnier and Prieur<sup>51</sup> maintained that the classic concept of the mechanical origin of biliary

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- 37. Tischner, R.: *Virchows Arch. f. path. Anat.* **175**:90, 1904
  - 38. Harley, V.: *Arch. f. Anat. u. Physiol.* **15**:291, 1893.
  - 39. Frey, in discussion on Stadelmann, E.: *Verhandl. d. Congr. f. innere Med.* **11**:115, 1892.
  - 40. Jagic, N.: *Beitr. z. path. Anat. u. z. allg. Path.* **33**:302, 1903.
  - 41. Abramow, S.: *Virchows Arch. f. path. Anat.* **181**:201, 1905.
  - 42. Naunyn, B.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **31**:537, 1919.
  - 43. Liebermeister: *Deutsche med. Wchnschr.* **19**:365, 1893.
  - 44. Minkowski: *Ztschr. f. klin. Med.* **55**:34, 1904.
  - 45. Sterling, S.: *Arch. f. exper. Path. u. Pharmakol.* **64**:468, 1911.
  - 46. Barron, E. S. G., and Bumstead, J. H.: *J. Exper. Med.* **47**:999, 1928.
  - 47. Hiyeda, K.: *Beitr. z. path. Anat. u. z. allg. Path.* **73**:541, 1925.
  - 48. Bloom, W.: *Bull. Johns Hopkins Hosp.* **34**:316, 1923.
  - 49. Scheunert, G.: *Beitr. z. path. Anat. u. z. allg. Path.* **86**:455, 1931.
  - 50. Haberland, H. F. O.: *Arch. f. klin. Chir.* **130**:248, 1924.
  - 51. Garnier, M., and Prieur, R.: *Presse méd.* **38**:1305, 1931.

retention in lesions of the common duct is incorrect, and that obstructive jaundice really represents a type of toxic or infectious jaundice. They believed that experimental ligation of the common bile duct brings about only transitory hyperbilirubinemia, incapable in itself of producing jaundice, which, when it develops later, they considered as always due to a superimposed toxic or infectious factor. Carrié<sup>52</sup> disagreed with this view, and Chabrol, Maximin and Busson,<sup>53</sup> while admitting that a secondary toxic factor may influence the production of jaundice in obstruction of the common duct, showed that in the beginning, at least, icterus is due to the mechanical factor of stasis. Following the induction of biliary obstruction, both the blood sinusoids and the lymph capillaries enter intimately into the mechanism of production of hyperbilirubinemia, the former being considered the most important by Whipple and Hooper,<sup>54</sup> whereas Barron and Bumstead<sup>16</sup> favor the latter. It is probable that in obstructive icterus the contents of the biliary conducting system are constantly being depleted by absorption and renewed by excretion from the polygonal cells, although not all of the bile entering the hepatic cells is necessarily excreted into the canaliculus, but may pass instead directly back into the perivascular tissue space, the blood stream or both.

Despite the fact that each of the three forms of necrosis occurring in the liver affected by stasis presents distinct and easily recognizable morphologic characteristics, no consistent attempt has been made to differentiate between them on the basis of pathogenesis and etiology. Charcot and Gombault<sup>5</sup> suggested that with increasing pressure in the canaliculi, rupture with extrusion of bile into the parenchyma results in destruction of hepatic cells, a view supported by Harley,<sup>38</sup> Frey,<sup>39</sup> Eppinger,<sup>11</sup> Abramow and Samoilowicz,<sup>13</sup> Carnot and Harvier<sup>11</sup> and Richardson.<sup>55</sup> Foà and Salvioli,<sup>56</sup> Pick<sup>57</sup> and Tsunoda,<sup>58</sup> finding no canalicular ruptures, considered the responsible factor to be the increasing pressure resulting from bile stasis. Gerhardt,<sup>36</sup> Richardson,<sup>55</sup> Ogata,<sup>15</sup> Rous and Larimore,<sup>59</sup> Hiyeda,<sup>47</sup> Rabl<sup>17</sup> and Kikuchi,<sup>60</sup> although recognizing the presence of canalicular ruptures, believed this to be of little importance in comparison with the chemical effects of stagnant

52. Carrié, P. A.: *Bull. méd., Paris* **45**:913, 1931.

53. Chabrol, E.; Maximin, M., and Busson, A.: *Presse méd.* **39**:892, 1931.

54. Whipple, G. H., and Hooper, C. W.: *Am. J. Physiol.* **42**:544, 1917.

55. Richardson, M. L.: *J. Exper. Med.* **14**:401, 1911.

56. Foà, P., and Salvioli, G.: *Arch. per le sc. med.* **2**:1, 1877.

57. Pick, E.: *Ztschr. f. Heilk.* **11**:117, 1890.

58. Tsunoda, T.: *Virchows Arch. f. path. Anat.* **193**:213, 1908.

59. Rous, P., and Larimore, L. D.: *J. Exper. Med.* **31**:609, 1920; **32**:249, 1920.

60. Kikuchi, S.: *J. Orient. Med.* **16**:12, 1932.

bile. Géraudel<sup>61</sup> looked on the lesions as representing a postmortem change, an idea refuted by Carnot and Harvier;<sup>14</sup> likewise, Jagic's<sup>40</sup> view that they arise from progressive inflammation was disproved. Vascular disturbances resulting from arterial thickening (Chambard<sup>33</sup>) or from portal constriction by dilating bile ducts (Tischner,<sup>37</sup> Judd and McIndoe,<sup>21b</sup> MacMahon, Lawrence and Maddock<sup>62</sup>), combined with the effects of toxic bile diffused (Janowski<sup>8</sup> and Loeffler<sup>63</sup>) or extruded from ruptures (Beloussow,<sup>64</sup> Ruppert<sup>7</sup> and Steinhaus<sup>65</sup>) in the canalicular system, are other factors which have been advanced to explain the causation of the necroses the development of which has been thought to be further enhanced by lack of nutrition and impaired regenerative ability of the remaining hepatic cells (MacMahon and Mallory<sup>20</sup>).

In our preparations focal midzonal necroses appeared at any time following obstruction of the common bile duct, being most common in the first two months, and having also been found in the sections of the liver of a patient dying three days after the appearance of jaundice. Vascular changes in the form of hypertrophy of the walls of the arteries and arterioles, thickening of the walls of the hepatic and central veins and sinusoids and tumor thrombosis of branches of the portal vein were observed. However, the sclerotic vascular changes are comparatively late developments in the liver affected by stasis and therefore cannot be held to account for the early appearing necroses, which are also frequently absent in many areas where portal vein and sinusoidal tumor thrombosis is a well marked feature. Vascular constriction by dilating bile ducts was not a particularly outstanding feature in our preparations, which, of course, were not especially designed to demonstrate this change. Moreover, the human body possesses remarkably finely adjusted compensatory mechanisms for overcoming resistance to the flow of blood (McNee<sup>66</sup>) although the structure and function of the circulatory system may vary somewhat in other animal groups (Cameron and Mayes<sup>67</sup>). The nature and efficiency of the collateral circulation in the dog and cat, and especially in man, are of the utmost importance. Numerous anastomoses exist, on the one hand, between the capillary plexuses of the hepatic arterial branches and those of the vessels entering the liver by way of the common bile duct and portal vein, and, on the other hand, between the terminations of the portal vein

• 61. Géraudel, E.: *J. de physiol. et de path. gén.* **8**:69, 1906.

• 62. MacMahon, H. E.; Lawrence, J. S., and Maddock, S. J.: *Am. J. Path.* **5**:631, 1929.

• 63. Loeffler, L.: *Virchows Arch. f. path. Anat.* **265**:41, 1927.

• 64. Beloussow, P. N.: *Arch. f. exper. Path. u. Pharmakol.* **14**:200, 1881.

• 65. Steinhaus, J.: *Arch. f. exper. Path. u. Pharmakol.* **28**:432, 1891.

• 66. McNee, J. W.: *Brit. M. J.* **1**:1017, 1068 and 1111, 1932.

• 67. Cameron, G. R., and Mayes, B. T.: *J. Path. & Bact.* **33**:799, 1930.

and hepatic artery. Conversely, a collateral circulation limited in its development and responding slowly to the stimulus of a deficiency in oxygen affords little help under the conditions of biliary stasis. Solowieff<sup>68</sup> and, more recently, Rous and Larimore<sup>69</sup> pointed out that occlusion of branches of the portal vein is merely attended by a progressive parenchymal atrophy.

The livers of rabbits and guinea-pigs excrete relatively large amounts of bile at comparatively low pressures, and at the beginning of stasis develop necroses which, however, are said to not appear in the livers of dogs in which animal the amount of bile secreted is small but the average pressure is high; the total quantity of bile produced is perhaps of more importance than the pressure at which it is secreted. Some investigators have claimed that human bile is innocuous and incapable of causing permanent hepatic damage, but if this is true, as Rous and Larimore<sup>69</sup> have pointed out, man differs from all other well studied animals in which, without exception, aseptic bile results in important local changes. The early observation that the degree of hepatic pigmentation is not paralleled by the development of necrosis is supported by the results obtained by Horrall,<sup>69</sup> Greene and Snell,<sup>70</sup> Horrall and Carlson<sup>71</sup> and Still,<sup>72</sup> who demonstrated bilirubin to be an inert substance in contrast to the bile acids, the toxic properties of which reside in the cholate radicle. By injecting fresh bile into the livers of rabbits and dogs, Hiyeda<sup>47</sup> produced areas of necrosis; Cameron and Oakley,<sup>73</sup> however, were unable to duplicate these observations in the rat even after ligation of the common bile duct had been performed with the idea of minimizing absorption of bile by the smaller ducts. If the focal midzonal areas of necrosis in the human being are due to the effects of extravasated bile, the quantity must be exceedingly small, for they show no pigmentation in excess of that found in adjacent hepatic cells. Furthermore, there are no transitional stages between the focal midzonal necroses and the biliary necroses which represent a true reaction of the tissue to extravasated bile. It is conceivable that coincident with early circulatory changes and increased intraductal pressure in the liver there is a lowering of oxygen tension resulting in nutritional disturbances of hepatic cells and in the production of intermediate metabolic products possibly of the nature of toxic amines. Furthermore, since the liver lies directly in the pathway of the portal blood carrying products absorbed from the

\* 68. Solowieff: *Virchows Arch. f. path. Anat.* **62**:195, 1875.

\* 69. Horrall, O. H.: *J. Lab. & Clin. Med.* **14**:217, 1928.

\* 70. Greene, C. H., and Snell, A. M.: *J. Biol. Chem.* **78**:691, 1928.

\* 71. Horrall, O. H., and Carlson, A. J.: *Am. J. Physiol.* **85**:591, 1928.

\* 72. Still, E. U.: *Am. J. Physiol.* **88**:729, 1929.

\* 73. Cameron, G. R., and Oakley, C. L.: *J. Path. & Bact.* **35**:769, 1932.



intestinal tract, the necrotic hepatic lesions may represent the effects of split protein products resulting from intestinal putrefaction due to the absence of bile.

The biliary necroses probably largely represent, as MacMahon and Mallory<sup>20</sup> have emphasized, the cellular reaction to inspissated bile, which, as a result of pressure, has escaped in the form of tubular masses from attenuated and ruptured ducts, remnants of which can often be recognized. These bile masses act as foreign bodies and are soon attacked by monocytes and foreign body giant cells, while still later fibroblasts proliferate about them and traverse their substance, giving them the appearance of old, partially organized thrombi. The bile mass undoubtedly contains toxic substances which are responsible for the enveloping necrotic zones which are always quite conspicuous when any part of the lobule is involved. Some of these lesions are probably also due to rupture of the canals of Hering and of the collecting tubules of Letulle, for the former are notoriously fragile even in normal preparations. This view is supported by the observation that fluid introduced under pressure into the common bile duct becomes extruded into the hepatic tissue in immediate relationship to the canals of Hering (Chrzonszczewsky,<sup>74</sup> Heidenhain,<sup>75</sup> Stern,<sup>76</sup> Beloussow<sup>64</sup> and Pfeiffer<sup>77</sup>) or more centrally within the lobule (Bürker<sup>25</sup>). Cameron and Oakley's<sup>73</sup> reconstructions in the rat demonstrate a constant close relationship between hepatic necroses and the interlobular bile ducts, although not a strict localization to the canals of Hering. According to Ribadeau-Dumas and Lecène,<sup>78</sup> the distribution of these lesions corresponds to the biliary lobules of Sabourin adjoining the portal canals.

Necrosis in the inner third of the lobule has been attributed to overloading of the cells with bile (Ogata<sup>15</sup>), which is thought to be secreted more abundantly in this area (Bürker<sup>25</sup>). The degree of pigmentation by bilirubin, which is nontoxic, is no indication, however, of the presence of toxic components of bile, although deleterious changes may conceivably ensue on actual physical overloading with this inert substance. There is abundant evidence suggesting the presence in the liver affected by stasis of a toxic factor in escaped bile which may affect chiefly the inner cells of the lobule, for when both central and peripheral cells are subjected to a known irritant under identical conditions, the former succumb much more readily and show the most marked degree of

• 74. Chrzonszczewsky, N.: *Virchows Arch. f. path. Anat.* **35**:153, 1866.

75. Heidenhain, R. P. H.: *Handbuch der Physiologie*, Leipzig, F. C. W. Vogel, 1883, vol. 5, p. 225.

76. Stern, H.: *Arch. f. exper. Path. u. Pharmakol.* **19**:39, 1885.

77. Pfeiffer, L.: *Arch. f. mikr. Anat.* **23**:22, 1884.

78. Ribadeau-Dumas and Lecène: *Arch. d. méd. expér. et d'anat. path.* **16**:191, 1904.

damage (Simonds and Callaway<sup>79</sup>). This vulnerability of the centrally located hepatic cells has been attributed to their greater functional activity and higher degree of specialization (Mallory<sup>80</sup>).

There was little or no attempt at regeneration. Multinucleated hepatic cells were rarely found, and mitotic figures and evidence of nuclear fission were not observed. Areas of destruction of the hepatic cells were gradually absorbed but were not replaced by new cells. These observations are in keeping with experimental data on the failure of restoration of the liver following obstruction of the common bile duct (Mann, Fishback, Gay and Green<sup>81</sup>).

Early in obstructive jaundice the proliferation of bile ducts and the increase in connective tissue are intimately related. As the newly formed bile ducts invade the peripheral portions of adjacent lobules they carry along a covering of connective tissue which enlarges the portal radicle. Steinhaus,<sup>65</sup> Gerhardt,<sup>36</sup> Chambard,<sup>33</sup> Foà and Salvioli,<sup>56</sup> Pick,<sup>57</sup> Rous and Larimore<sup>59</sup> and Richardson<sup>55</sup> attributed the changes in the bile ducts to the irritation arising from increased pressure within them. Charcot and Gombault,<sup>5</sup> Nasse<sup>82</sup> and Ogata<sup>15</sup> considered the two processes to be essentially independent although brought about by the same cause, namely, the irritation from static bile. We agree with Harley and Barratt,<sup>83</sup> Loeffler<sup>63</sup> and Paolini<sup>84</sup> that the changes in the bile ducts and connective tissue are probably brought about, at least in part, by a combination of both of these factors. As the pressure within the bile ducts increases there are a coincident stretching and attenuation of their walls, and bile diffuses into the surrounding tissue, where it actively stimulates hyperplasia of the connective tissue (Richardson<sup>55</sup> and Rous<sup>28</sup>), which is only slight or moderate within the first two months but then proceeds independently of the changes in the bile ducts. It is possible that the damaged hepatic cells form intermediate metabolic products the action of which also stimulates the growth of connective tissue. A number of investigators advanced the view that the proliferation of the connective tissue depends largely on the organization of necrotic foci (Beloussow,<sup>64</sup> Foà and Salvioli,<sup>56</sup> Gerhardt,<sup>36</sup> Janowski,<sup>8</sup> van Heukelom,<sup>85</sup> Tsunoda,<sup>58</sup> Purpura<sup>86</sup> and Fabris<sup>87</sup>). In our cases, the focal midzonal necroses showed

79. Simonds, J. P., and Callaway, J. W.: *Am. J. Path.* **8**:159, 1932.

80. Mallory, F. B., cited by Simonds and Callaway.<sup>79</sup>

81. Mann, F. C.; Fishback, F. C.; Gay, J. G., and Green, G. F.: *Arch. Path.* **12**:787, 1931.

82. Nasse, D.: *Semaine méd.* **16**:202, 1894.

• 83. Harley, V., and Barratt, W.: *Brit. M. J.* **2**:1743, 1898.

• 84. Paolini, R.: *Riforma med.* **43**:843, 1927.

85. van Heukelom, S.: *Beitr. z. path. Anat. u. z. allg. Path.* **20**:221, 1896.

86. Purpura, F.: *Sperimentale, Arch. di biol.* **69**:819, 1915.

• 87. Fabris, A.: *Arch. per le sc. med.* **31**:429, 1907.

no attempt at organization, although the reactive and healing processes about the biliary necroses did contribute to the new formation of connective tissue at a time, however, when that resulting from other causes was already well established.

#### SUMMARY

With obstruction to the common bile duct by carcinoma of the head of the pancreas, the biliary conducting system undergoes a tremendous volumetric increase, with corresponding stretching and thinning of its walls. The process extends uniformly into the branches of the fourth and fifth orders, although the dilatation may be more marked in the radicles of the left hepatic lobe by virtue of the lessened amount of parenchyma in that portion of the liver and also possibly because of the course and relationship of the left hepatic duct and its corresponding artery and vein. The vasa aberrantia and parietal sacculi become effaced. The contents of the biliary conducting system in the beginning consist of dark, thick, ropy, inspissated bile which later tends to become pale and thinner. The canaliculi are distended with bile thrombi, many of which remain in situ, although others are extruded into the tissue spaces and sinusoids, where they may be phagocytosed by Kupffer cells and macrophages. Bile pigment in the form of granules or droplets and in colloidal suspension may be found in varying amounts, chiefly in the cells of the central portions of the lobule. Regressive lesions are present as mild and localized degenerative and necrotic changes involving the hepatic cells about the central vein, as nonpigmented focal midzonal areas of necrosis and as deeply pigmented biliary necroses occurring either in the outer portion of the lobule or within the portal radicle. The intrahepatic ducts elongate and become tortuous, and they show, in addition, a true proliferation. A new formation of connective tissue occurs simultaneously with the changes in the bile ducts until after the second month, when a rapid, independent and progressive proliferation takes place, resulting in a well marked deposit of connective tissue which is interlobular, intralobular and, in long-standing cases, even perilobular in distribution. The hepatic lobules show a corresponding reduction in size without much architectural alteration except at the peripheries, where atrophy may become well marked. The walls of the branches of the hepatic artery undergo hypertrophy, and those of the branches of the hepatic vein and to a less extent of the portal veins show a new formation of fibrous tissue. The liver possesses little or no regenerative ability in the face of total stasis.

# INFLUENCE OF EXTRACT OF ANTERIOR PITUITARY ON AUTOTRANSPLANTED AND HOMEO- TRANSPLANTED THYROID

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Loeb and his collaborators<sup>1</sup> showed that extracts of the anterior lobe of the pituitary gland stimulate the growth of the thyroid. This effect is especially marked in guinea-pigs. These observations were confirmed by many other investigators.<sup>2</sup>

In another series of investigations, Loeb<sup>3</sup> analyzed the reactions of the host against autotransplants, syngenesiotransplants, homeotransplants and heterotransplants. Definite relations were found to exist between the intensity of the lymphocytic and connective tissue reactions on the part of the host and the genetic relationship of the host and the donor. While autotransplantation in general makes possible the normal development of the graft, homeotransplantation, as a rule, leads to severe injury or complete destruction of the transplant.

It seemed of interest to investigate the behavior of the various types of transplants under the influence of extract of the anterior lobe of the pituitary gland of cattle. The thyroid gland represents an especially favorable material for such investigations. In particular did I wish to answer the following questions:

1. Under the influence of the extract does the transplant change similarly to normal thyroid tissue?
2. Does this change have any effect on the resistance of the transplant to the direct and indirect action of homeotoxins?

I therefore carried out two series of experiments on guinea-pigs; in the first, the effect of this extract on the growth of autotransplants was studied, and in the second, its effect on the reactions of the host against homeotransplants of thyroid tissue and on the preservation of

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1. Loeb, L.: *Klin. Wchnschr.* **11**:2121, 1932. Loeb, L., and Bassett, R. B.: *Proc. Soc. Exper. Biol. & Med.* **26**:860, 1929; **27**:490, 1930. Loeb, L.; Bassett, R. B., and Friedman, H.: *ibid.* **28**:209, 1930.

2. Oehme, C.; Poal, H., and Kleine, H. O.: *Klin. Wchnschr.* **11**:1449, 1932. Schittenhelm, A.: *Deutsche med. Wchnschr.* **58**:803, 1932. Silberberg, Martin: *Krankheitsforschung* **8**:171, 1930; *Proc. Soc. Exper. Biol. & Med.* **26**:166, 1929; *Virchows Arch. f. path. Anat.* **289**:201, 1933.

3. Loeb, L.: *Am J. Path.* **2**:99 and 315, 1926; **3**:251, 1927; *J. Cancer Research* **10**:252, 1926; *Am. Naturalist* **54**:45, 1920; *J. Gen. Physiol.* **8**:417, 1926; *Endocrinology* **13**:49, 1929; *Physiol. Rev.* **10**:547, 1930; *Biol. Bull.* **40**:143, 1921.

the latter. Altogether, seventy-four animals were used in these experiments. I shall describe only certain representative experiments among a larger number which I have carried out.

I used acid extract of the anterior lobe of the pituitary gland prepared in a similar way to that used in my previous investigations, except that a small piece of camphor was added to the extract as a preservative. One and one-half cubic centimeters of this extract was injected daily into guinea-pigs intraperitoneally. Young guinea-pigs weighing on the average from 150 to 200 Gm. were used, and the changes in weight of the animals were determined each day before feeding. The experiments were carried out mainly during the autumn and winter. As a rule, one lobe of the thyroid gland was transplanted into a subcutaneous pocket in the abdominal wall of the host animal, with aseptic precautions, under narcosis with ether.

At the conclusion of the experiment, the transplant was taken out together with the surrounding host tissue and fixed in a trinitrophenol-formaldehyde solution.<sup>4</sup> This material was embedded in paraffin and cut in serial sections, which were stained with hematoxylin and eosin.

#### OBSERVATIONS

I. *Effect of the Extract on the Normal Thyroid* (six experiments).—As described by various investigators, daily injections of from 1 to 3 cc. of this extract produced an enormous increase in the size and number of cells of the acinar epithelium, the proliferation taking place by means of mitoses. The first changes were found after one injection. The strongest effect, however, was seen on an average after from six to ten injections: The formation of large papillary excrescences protruding into the lumen of the acini, migration of phagocytes into the colloid, softening, liquefaction and perfect absorption of the colloid were observed. Under these conditions, the thyroid assumed the appearance of that characteristic of exophthalmic goiter.

II. *Effect of the Extract on the Autotransplanted Thyroid*.—1. Controls (eight experiments): After autotransplantation of the normal gland, the peripheral part of the thyroid remained alive, while the insufficiently nourished central portion became necrotic and was replaced by dense connective tissue. In the living part as well as in the adjoining portion of the central necrotic zone, there was a new formation of blood vessels. Fibroblasts and histiocytes grew into the central necrotic mass and absorbed it. Within about three weeks, regeneration of the acini was taking place from the peripheral zone, and the transplant more and more assumed the appearance of the normal thyroid. The colloid was hard. In some cases a loose, well vascularized connective tissue was found in the center of the transplant instead of acini. The surrounding tissue likewise consisted of a more or less loose connective tissue, which was well vascularized.

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4. This solution is composed of: solution of formaldehyde, U. S. P., 500 cc.; distilled water, 1,500 cc.; glacial acetic acid, 100 cc.; trinitrophenol to saturation point.

2. Autotransplanted Thyroid: GROUP 1.—In this group autotransplantation of the thyroid gland was followed by a series of injections of the extract with and without intervening periods of rest. In other experiments, the series of injections was either preceded or followed by a period of rest, during which no injections were given.

(a) Autotransplantation, followed by six injections of extract, one being given daily (two experiments). The transplanted thyroid showed hypertrophy and hyperplasia. The effect of the extract was evident in a larger number of mitoses, the increased size of the acinar epithelium, the presence of some phagocytes in the acini and a softening and liquefaction of the colloid. A considerable increase in the number of acinar cells had taken place. It was interesting that in comparison with the controls, the central portion of the transplant seemed to be better preserved, indicating a greater resistance of this tissue.

(b) Autotransplantation, followed by six injections of extract and a subsequent period of rest. In the transplants which were removed six days after the last injection (two experiments), the effect of the extract on the transplant as well as a good regenerative growth were still observed. But the acinar epithelium had become lower and the number of the mitotic figures was diminished; the thyroid transplant was on the way to resuming the structure of the normal, resting gland.

After twelve days of rest (two experiments), signs were still present of the preceding period of hypertrophy in the transplanted tissue, which had healed in well; the regenerative growth had been very good. The acini were increased in number; they were still enlarged and contained again hard, newly formed colloid. The periphery was surrounded by loose connective tissue; a central fibrous knob was not visible.

(c) Autotransplantation, followed by a period of rest lasting five days, then by six subsequent injections of extract (four experiments). The transplant was very well preserved. The formerly necrotic central zone had been replaced by dense fibrous tissue. In the peripheral area regeneration was noticeable in addition to the typical hypertrophy caused by the extract.

2. Transplantation, followed by a period of rest lasting fifteen days, then by ten subsequent injections, one being given daily (two experiments). In this case in which the injections were given after a longer period of rest following transplantation, at a time therefore when the transplant had regained the structure of the normal thyroid, the effects of the extract were the same as in the normal thyroid. The graft showed a hypertrophic acinar epithelium, increase in mitotic proliferation and liquefaction or complete absorption of the colloid.

3. Transplantation, followed by a period of rest lasting eighteen days, then by a series of six injections (two experiments). Under the conditions of this experiment the hypertrophy was more marked than under those of the preceding experiment. Figure 1 shows the typical structure. The thyroid was well healed in and was surrounded by a small amount of connective tissue and large blood vessels. In the transplant itself marked hypertrophy was observed. The cells of the acinar epithelium were large and high; there was an increased number of mitoses, and the colloid had become soft and had mostly been absorbed; owing to the increase in the number of acini, the interacinar connective tissue had almost completely disappeared.

Summary of series c: The influence of the extracts on the transplants was striking. If a period of rest was maintained between transplantation and the beginning of the injections, the hypertrophy of the acini was more marked than

in those experiments in which injections followed the transplantation directly. However, the difference between these two types of experiments is only of a quantitative character.

(d) Transplantation, followed by a period of rest lasting six days, then by six injections of extract, followed by a second period of rest. The only difference between the results in this group of experiments and those in the preceding group consisted in the beginning disappearance of the effects of the extract when injections were omitted for some time previous to examination. This applies especially to the character of the colloid; a new formation of hard colloid had set in under these conditions. However, an increase in size and number of the acini was still

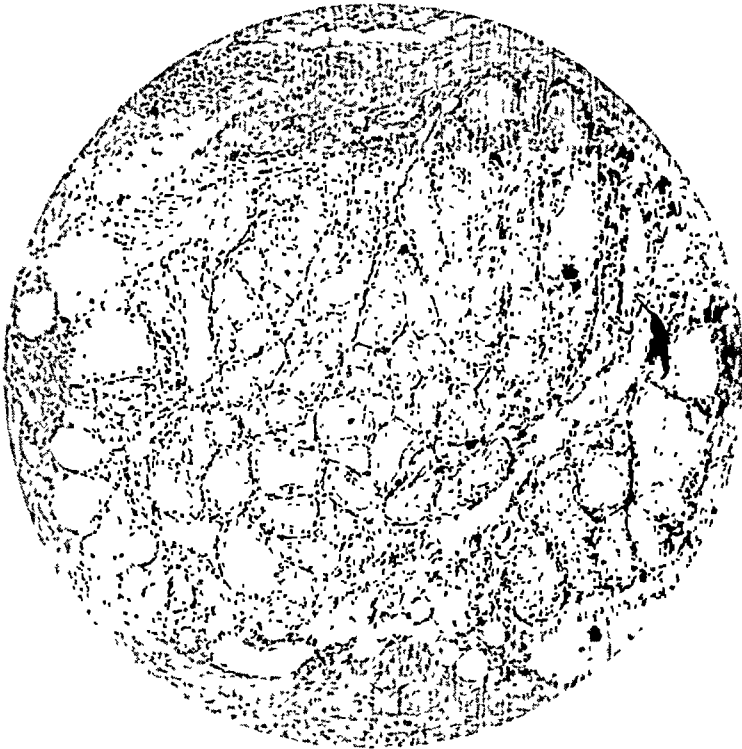


Fig. 1.—Autotransplantation was followed by eighteen days of rest and six injections of extract of the anterior lobe of the pituitary gland. The acini are free from colloid, the epithelium enlarged and a capsule of connective tissue surrounds the transplant. Medium magnification.

noticed, but the epithelium had become lower and the mitotic figures were diminished in number. The thyroid was therefore in the process of returning from a state of hypertrophy to that of rest.

GROUP 2.—In this group, transplantation was preceded by a series of injections of extract of the anterior lobe of the pituitary gland; subsequent to transplantation there was a period of rest.

(a) Six injections of extract, followed by transplantation and a six day period of rest (six experiments). The central portion of the transplant was necrotic, while the peripheral part of the gland was preserved. The surrounding loose fibrous tissue contained a few fibroblasts and blood vessels. Under high power magnification one could still notice a certain effect of the extract, as the colloid was softened

and contained some phagocytes, and many mitotic figures were seen, but the acinar epithelium had already become lower and was in the process of returning to a state of rest.

(b) Ten injections given previous to transplantation, with a period of rest lasting ten days between transplantation and examination (two experiments). During the ten day period of rest a return of the graft to a normal condition was noticeable; the phagocytes had disappeared and though there was still an increased number of mitoses, the acinar epithelium had become flatter.

(c) Six injections, followed by transplantation and a period of rest of eighteen days. In this transplant, the necrotic part had been replaced by a dense fibrous tissue. From the peripheral remnant of the thyroid, regeneration had developed. The changes, due to a preceding stimulation of growth by the extract, had disappeared. A new formation of hard colloid in the well formed acini had taken place.

GROUP 3.—A series of injections was followed by transplantation; the graft was then subjected to a new series of injections. The transplant was removed either directly after the last injection or following a period of rest subsequent to the second period of injections.

(a) Six injections, followed by transplantation and a second series of six injections (four experiments). A small central area of the transplant was still partly necrotic, but its organization by fibrous tissue had progressed. The periphery was well formed and showed large acini with hypertrophic cells. The colloid was softened or entirely liquefied. The softened colloid might have contained phagocytes. Regeneration, as usual, had started from the peripheral living thyroid tissue.

(b) Six injections, followed by transplantation, then a second series of eighteen injections (two experiments). The central, formerly necrotic zone of the thyroid was replaced by a dense fibrous tissue. In the periphery the stimulation was indicated by the finding of a large number of mitoses, but the stimulation was not so marked as after six injections, as indicated by the lower acinar epithelium in comparison with the earlier stage. In the acini only a few phagocytes were visible.

(c) Six injections, followed by transplantation, then a second series of six injections. The thyroid was examined following a period of rest lasting eighteen days. The regeneration of thyroid tissue had made good progress, but the acinar epithelium was in the process of returning to the resting stage, as indicated by new formation of hard colloid.

(d) Six injections followed by transplantation, then by an eighteen day period of rest and a second series of six injections (two experiments). The regeneration was completed. The peripheral acini were distended and showed the structural changes characteristic of the extract. However, the growth was not so good as in those cases in which the animal did not receive injections before transplantation.

III. *Effect of the Extract on the Homeotransplanted Thyroid.*—After determining the effect of the extract of the anterior lobe of the pituitary gland on the autotransplanted thyroid, it was of interest to determine the effect of this substance on the homeotransplanted gland. The fate of the homeotransplanted thyroid gland differs greatly from that of the autotransplanted tissue. The various processes leading to the destruction of the former have been analyzed by Hesselberg and Loeb. Some control experiments were first carried out in which homeotransplantation of the thyroid gland of the guinea-pig was carried out in order to obtain a standard with which to compare the behavior of the homogeneous graft under the influence of extracts of the anterior lobe of the pituitary gland.



1. Controls (four experiments): Connective tissue grew in greater quantity into the central necrotic part of the homeotransplanted than of the autotransplanted gland, and it destroyed the peripheral, living, glandular zone, first causing a shrinkage and gradual obliteration of the acinar lumens. Seven or eight days after transplantation, a remarkable infiltration of the transplant with lymphocytes developed and helped in the destruction of the transplant. Usually from twenty to thirty days after transplantation the graft was completely destroyed. As a rule, homeotransplants are preserved for only a short time. While regenerative processes may be found also in homeotransplants, they are generally more extensive in autotransplants. Twenty-four days following homeotransplantation, a perfect healing in of thyroid tissue or a new formation of acini with colloid such as was seen in the autotransplanted glands had not taken place.

Furthermore, it may be noticed that it is nearly always the lymphocytes which mainly cause the elimination of the homeotransplant, while in autotransplants these cells are present only in a very small number, and in later periods they are not more frequent than in the normal gland.

2. Experiments with Extract: GROUP 1.—Homeotransplantation was followed by injections of extract.

(a) Homeotransplantation, followed by eighteen injections of extract, then removal of the transplant for microscopic examination (two experiments). The peripheral part, as well as the center of the transplant, showed preserved thyroid tissue. The surrounding area consisted of relatively dense fibrous tissue. A regeneration of acini had taken place. It was especially noted that a mass of lymphocytes penetrated the transplant without, however, completely destroying it. The effect of the extract was clearly indicated by the high acinar epithelium, the large number of mitotic figures and the softened or liquefied colloid; the change in the homeotransplant was, therefore, similar to that called forth in the normal gland by the injection of the extract. However, after eighteen injections the normal thyroid does not usually show so strong an influence of the extract as did this transplant.

(b) Homeotransplantation, followed by a series of eight injections, then by a ten day period of rest (two experiments). The principal difference between this and the former experiment was that here only very few acini were preserved. The main part of the transplant was destroyed by lymphocytes; a newly formed capsule of connective tissue was not observed. The only part of such transplants preserved may consist of a few scattered acini. In another similar experiment the whole transplant had been destroyed.

(c) Transplantation, followed by a period of rest lasting five days, then by a series of twelve injections, one being given daily (two experiments). In this experiment the whole transplant was well preserved and enclosed in a dense fibrous capsule in one animal. In the periphery was found excellently regenerated thyroid tissue in which some acini contained colloid. In the center some lymphocytes were found, which, however, did not cause much destruction. Under high power magnification the effect of the extract was recognized by the presence of high acinar epithelium, an increase in the number of mitotic figures and the liquefaction of the colloid containing a few phagocytic cells. However, as far as the regeneration of the transplanted tissues in the two animals used in this case was concerned, considerable variations were found; while one of the transplants was well preserved, the other did not show any growth, although both pieces had been left in their respective hosts for the same length of time and an exchange of thyroids had taken place between the two animals.

(d) Transplantation, followed by a five day period of rest, then a series of six injections followed again by a seven day period of rest (two experiments). The center of the transplant consisted of a small amount of connective tissue with many lymphocytes, but also in this case the transplant was, on the whole, well preserved. The effect of the extract was diminishing: the epithelium of the acini became lower and the colloid accumulated again in the acini. In the periphery some lymphocytes were seen, and the surrounding area consisted of fibrillar connective tissue and blood vessels. There were no homeotoxic cells, which destroy the transplant. Growth and regeneration in this experiment were very good and similar to those seen in series *c* (fig. 2).

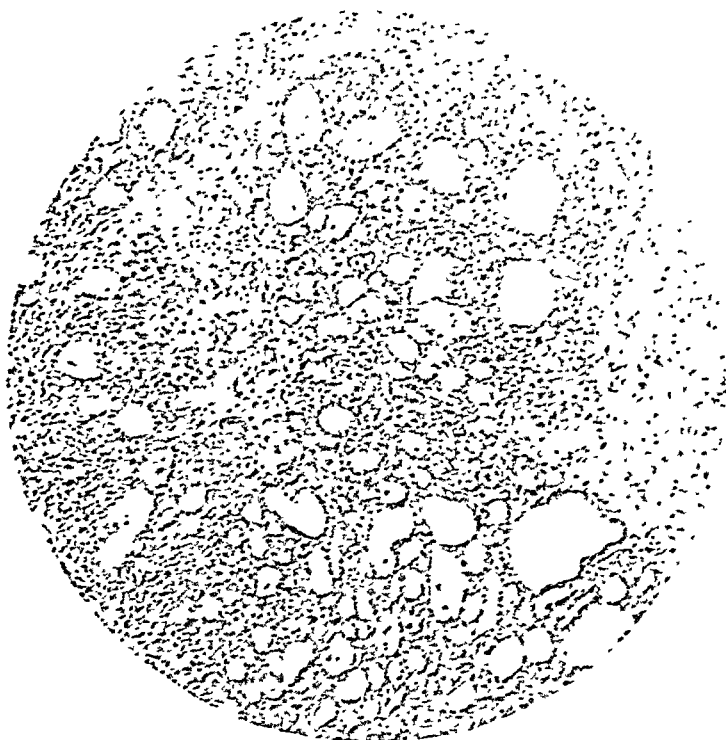


Fig. 2.—Homeotransplantation was followed by five days of rest and six injections, and a second period of rest lasting seven days. Connective tissue and enlarged acini are seen. Medium magnification.

GROUP 2.—A series of six injections was given to the donor of the transplant preceding the transplantation. As a result of these injections, the thyroid to be transplanted had become hypertrophic. The hypertrophic gland was then transplanted. The graft was removed at the end of the experiment, either following a period of rest or after a series of injections had been given to the host, so that the transplant was subjected altogether to the influence of two series of injections of extract, one being given before transplantation and a second one following transplantation.

(a) Six injections given to the donor; transplantation of the hypertrophic gland, followed by a period of rest of eighteen days (two experiments). In this series only small remnants of the transplants were found. Almost the whole thyroid transplant was destroyed and replaced by connective tissue containing a large

number of lymphocytes. No difference between these pieces and the control homeotransplants could be seen.

(b) Six injections given to the donor; transplantation, followed by six injections into the host (three experiments). The transplant was necrotic in the center. A loose fibrous tissue developed here, and lymphocytes began to invade the transplant. There was no essential difference between the results of these experiments and those of the controls.

(c) Six injections given to the donor; transplantation; six injections into the host followed by twelve days of rest (two experiments). The results are the same as in experiment *b*. The graft was completely destroyed by lymphocytes. No regeneration had taken place, nor was an effect of the extract noticeable (fig. 3).

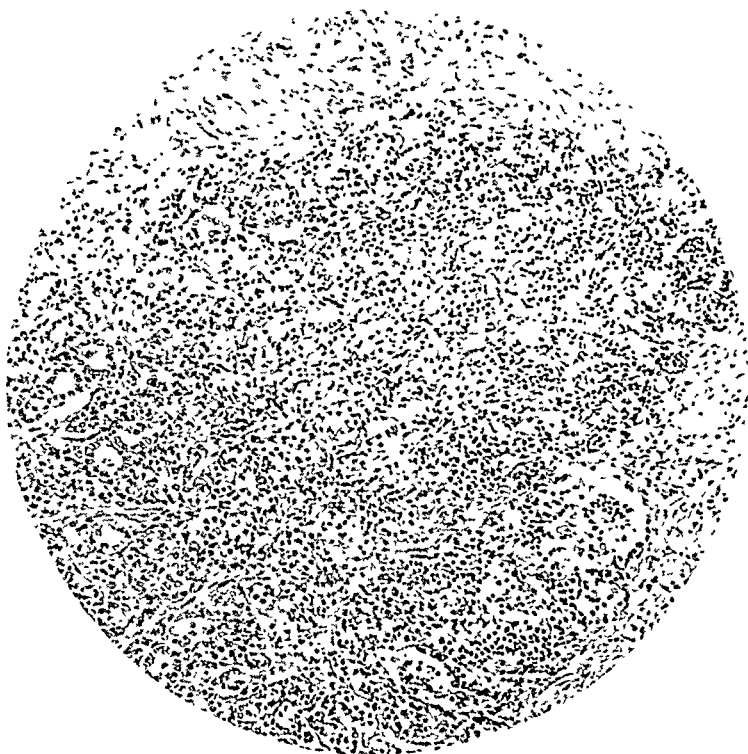


Fig. 3.—The thyroid used for homeotransplantation was taken from a donor which had received six injections with the extract. The new host received six injections; after a subsequent period of rest lasting twelve days, the homeotransplant was examined. The greater part of the graft has been destroyed. Medium magnification.

(d) Six injections given to the donor; transplantation, followed by eighteen injections into the host (two experiments). Under the conditions of this experiment the graft was likewise completely destroyed by homeotoxic lymphocytes.

A number of other similar experiments had analogous results.

#### COMMENT

My results are in agreement with the conclusions of Loeb and of Borst,<sup>5</sup> who showed that the thyroid gland represents a relatively resistant tissue and that autotransplantation of this organ is successful.

5. Borst, M.: Brit. M. J. 2:383, 1913.

They agree also with the view that the relations between the genetic constitutions of the host and the donor, as expressed in the individuality differentials, determine the result of transplantation. My experiments have shown that the transplanted organ responds to the stimulus of the hormone of the anterior lobe of the pituitary gland in approximately the same manner as does the normal organ, and this applies to the autotransplant as well as to the homeotransplant, although in the case of the latter the amount of tissue that remains alive is much smaller and the parts that remain alive are exposed to the injurious reactions of the host. As Loeb has shown, compensatory hypertrophy may develop in autotransplanted as well as in homeotransplanted thyroids. Likewise, retrogression of the hypertrophic and hyperplastic changes in the transplants following the cessation of injections of the extract of the anterior lobe of the pituitary gland takes place in the same manner in the transplants as in the normal gland which has been left in situ. Thus, while the transplants are affected by the extract in a similar way to the normal gland, the hypertrophic and hyperplastic changes may not be quite so marked in the transplants, perhaps owing to the relatively unfavorable conditions which are present in the graft during the period following transplantation. A summation of the stimulus to regenerative growth which acts on the transplant, owing to its separation from its normal environment, and of the effect of the extract does not take place.

The result is different when compensatory hypertrophy is induced in one of the lobes of the thyroid gland through extirpation of the other lobe: if extract of the anterior lobe of the pituitary gland is allowed to act on thyroid tissue which is in process of compensatory hypertrophy, a summation of the two effects, compensatory hypertrophy and hypertrophy and hyperplasia induced by the extract, does occur, presumably because under these conditions the nontransplanted tissue is in a satisfactory state and can readily respond to the stimulating agent.

As stated, extract of the anterior lobe of the pituitary gland can exert a stimulating effect also on the homeotransplanted thyroid gland, and it is probably due to the increased growth energy induced in the transplant by the hormone of the anterior lobe of the pituitary gland that the thyroid tissue is able to maintain itself better than the ordinary homeotransplanted gland and to overcome to some extent the injurious action of the lymphocytes of the host. In order to accomplish this result, the injections of the extract must be begun at an early period following transplantation; otherwise, if the injections are started at a time when the reaction against the transplant has become manifest, the stimulation by the hormone of the anterior lobe of the pituitary gland is without avail. If, in some cases, the graft is destroyed at an early period, notwithstanding the large doses of extract given, one may assume

that the difference in the constitution of the individuality differentials of host and transplant was so great that it could not be overcome by the added growth momentum of transplanted tissue.

One might suppose that the regenerating tendency of thyroid tissue, which had been exposed to the influence of the extract previous to transplantation, would be very strong, but, under these conditions, the tissue to be transplanted is already hypertrophic, and hypertrophic thyroid tissue seems unable to withstand the injurious effects of either autotransplantation or homeotransplantation as readily as normal thyroid gland. One may assume that the hypertrophic tissue is much more active, as far as metabolic changes and growth are concerned, than the ordinary thyroid gland, and, furthermore, that it needs a greater amount of oxygen than can be supplied during transplantation; thus it is injured to a greater extent than is the normal thyroid gland. One finds, therefore, that whereas administration of extract of the anterior lobe of the pituitary gland following transplantation exerts a beneficial effect on the transplanted tissue, administration of this extract previous to transplantation is very injurious and leads to more unfavorable results than transplantation of the normal organ.

#### SUMMARY

1. Injection of extracts of the anterior lobe of the pituitary gland may induce hypertrophic and hyperplastic changes not only in the normal, but also in the transplanted, thyroid gland of the guinea-pig; it may be effective not only in the autotransplanted, but also in the homeotransplanted, gland. However, the effect is usually less marked in the transplant than in the normal tissue.

2. The stimulation of the homeotransplanted thyroid tissue by the hormone of the anterior lobe of the pituitary gland may increase the ability of the graft to resist the injurious factors which are active after homeotransplantation; the preservation of the graft is thus improved. In order to accomplish this effect, it is necessary to apply the extracts after the thyroid has been transplanted into the strange host.

3. If, however, the tissue is caused to grow and to undergo hypertrophic changes previous to transplantation, the thyroid tissue shows a diminished power of resistance and is destroyed at an early period following transplantation. One must assume that the stimulated thyroid gland is less suitable for transplantation than the resting normal gland.

# General Review

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## MELANIN

### II. A REVIEW OF CHEMICAL ASPECTS OF THE MELANIN PROBLEM

VICTOR C. JACOBSEN, M.D.

ALBANY, N. Y.

The classification of the many colored materials found in human tissues in health and in disease presents problems which have been approached from various angles over a long period of years. The problems as a whole are similar for the lower orders of the animal kingdom as well, and possibly even for the protozoa. The medical student is taught that the pigmented substances of the body are divided into two main groups, the endogenous and the exogenous. The former consist of the pigments derived from hemoglobin, such as hemosiderin, hematoïdin, hemofuscin and the bile pigments, the rather indefinite group of lipochromes and melanin. The exogenous pigments come from inhaled dust, producing the pneumonokonioses, from tattooing and from foods and medicinal substances, causing carotenemia and argyria, for instance.

Melanin has generally been regarded as strictly endogenous, but since it is found in foods of plant and animal origin, it has also, at least theoretically, these exogenous sources. The term melanin is derived from the Greek μέλας, meaning black. The pigment is black, however, only when viewed in large amounts. Smaller deposits are brownish, and hence fuscin would be more appropriate etymologically. In the medical literature are found such expressions as melanism or melanosism, meaning varying degrees of melanin deposit in the skin; melanocyte, a cell containing melanin; melanoma, a tumor containing melanin, and melanuria, the presence of melanin in the urine.

Melanin is found in the pigment of the hair, the skin, the choroid of the eye, the ciliary body, the pigment layer of the retina and various nerve cells. There are analogous foci of coloration in the lower animals, the "ink" of the squid and butterfly wings; in fact, the entire animal kingdom uses this pigment in its manifold color patterns and for protection

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From the Department of Pathology, Albany Medical College.

This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

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by color mimicry against environmental perils. The amount of melanin necessary to color tissues grossly is very small. It has been estimated that there is only 1 gram in the entire skin of a Negro (Abel and Davis<sup>1</sup>). In a liver affected with metastatic melanoma, Berdez and Nencki<sup>2</sup> obtained 300 Gm. of melanin and estimated 500 Gm. in the entire body. Helman<sup>3</sup> computed that melanin may constitute 7.3 per cent by weight of the fresh substance of some melanomas. The smallest amount of melanin or its total absence is seen in the albino, a congenital condition which occurs as a mendelian recessive character. Acquired loss of melanin usually occurs in localized areas of the body, the best example of this being vitiligo. However, in alopecia areata, leprosy, tuberculids, syphilids and burns, the lesion is often associated if not in the most active stage, at least in the healing and healed lesions, with partial or complete loss of the pigment in the skin and in the hair. Indeed, it seems that every disease of the skin has an effect on its melanoblasts and melanophores, but the effect may be either loss of pigment or a great increase and is dependent on factors little understood.

Increased melanin deposits are seen in a variety of conditions. The normal melanin content varies with the races, with racial subdivisions and even individually, so that no color standard is possible. From ordinary "tanning" and the freckle or lentigo, chloasma or liver spots to xeroderma pigmentosum, pigmented papillomas and the pigmented nevus, there is at the other end of the spectrum the very dangerous tumor melanoma malignum. As expressions of serious disease involving the pigment regulatory system there are Addison's disease, Recklinghausen's disease (neurofibromatosis), arsenic poisoning and senility, in which diffuse melanosis cutis is often a striking feature. Endocrine disturbances affecting especially the anterior lobe of the hypophysis and the thyroid are often accompanied by excessive pigmentations.

A substance with so definite a name which has adhered for these many years naturally has been studied to determine its chemical composition. The greatest difficulty in the way of such an investigation is its relative insolubility in fluids from which it could be extracted in pure form through crystallization. Melanin can be removed from tissues with alkalis and to a less degree by acids, but while the resultant solution is highly colored it cannot be assumed that the extracted material is any longer simply melanin. Nevertheless, Hoffmeister<sup>4</sup> endeavored to

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1. Abel, J. J., and Davis, W. S.: *J. Exper. Med.* **1**:361, 1896.

2. Berdez, J., and Nencki, M.: *Arch. f. exper. Path. u. Pharmacol.* **20**: 346, 1886.

3. Helman, D.: *Zentralbl. f. inn. Med.* **23**:1017, 1902.

4. Hoffmeister, cited by Wells, H. G.: *Chemical Pathology*, Philadelphia, W. B. Saunders Company, 1925, p. 527.

establish its molecular composition and arrived at the proportion  $N:H:C=1:5:5$ . Sulphur was high, about 10 per cent in melanomas and 12 per cent in the ink of the squid. No sulphur was found in choroid melanin. The sulphur in hair was from 2 to 4 per cent. A trace of iron was sometimes found. Salkowski<sup>5</sup> mentioned several melanins all of which contain sulphur and phosphorus. As stated by Matthews,<sup>6</sup> the composition of melanin is not definitely known, and he regards it as a not well defined chemical substance.

Gortner,<sup>7</sup> who has studied plant and animal pigments for many years, gave the following composition for melanin: C, 52.57; H, 7.28; N, 13.43; S, 1.33, or, comparing his findings with Salkowski's ratio of  $N:H:C=1:5:5$ , the proportions would be  $N:H:C=2:1:7.5$ . This divergence probably is due to the different sources of the pigments used. It does not, however, prove that there is not a basic pigmented component of the complex biologic unit called melanin.

Gortner<sup>8</sup> isolated from black wool by treatment with extremely dilute alkalis a material of protein nature which was intensely black but which on acid hydrolysis yielded about 90 per cent of its weight in amino-acids. This complex he regarded as a conjugated protein in which melanin is the associated chromogen. He was unable to isolate a similar compound from nonpigmented wool, and since other pigmented structures like human hair, black rabbit fur and black feathers did not yield a similar product, he thought it reasonable to believe that the pigment of black wool exists in the form of melanoprotein rather than as melanin unassociated with the protein molecule. Spiegler,<sup>9</sup> on the other hand, isolated from white wool a white chromogen closely related to melanin chemically.

The animal pigment melanin is now generally regarded as formed by the interaction of a chromogen and an oxidase which is apparently tyrosinase.

In 1896 Bertrand<sup>10</sup> discovered an oxidase which produced various colors to a black precipitate with tyrosine and called it tyrosinase. In 1902 von Fürth and Schneider<sup>11</sup> suggested that melanins were probably the product of the interaction of tyrosine and tyrosinase. In the same year Dewitz<sup>12</sup> found that pigmentation of the fly, *Lucilia*

5. Salkowski, E.: *Virchows Arch. f. path. Anat.* **240**:353, 1922.

6. Matthews, A. P.: *Physiological Chemistry*, New York, William Wood & Company, 1925, p. 710.

7. Gortner, R. A.: *J. Biol. Chem.* **8**:341, 1910.

8. Gortner, R. A.: *Biochem. Bull.* **1**:207, 1911.

9. Spiegler, E.: *Beitr. z. chem. Physiol. u. Path.* **4**:40, 1902; **10**:253, 1907.

10. Bertrand, G.: *Compt. rend. Acad. d. sc.* **122**:1215, 1896.

11. von Fürth, O., and Schneider, H.: *Beitr. z. chem. Physiol. u. Path.* **1**:229, 1902.

12. Dewitz, M. I.: *Compt. rend. Soc. de biol.* **54**:44, 1902.

caesar, was caused by the action of tyrosinase on a chromogen. In 1900 Roques<sup>13</sup> showed that a similar reaction occurred during metamorphosis and pigmentation of a beetle, *Limnophilus flavicornis*, Fabr., and in 1905 Phisalix<sup>14</sup> noted similar changes in the integument of the cockroach larva, *Phyllodromia germania*. In 1904 Durham<sup>15</sup> extracted the fetal skins of black rabbits and agouti guinea-pigs, and after incubating at 37 C. for ten days in the presence of tyrosine and ferrous sulphate dark precipitates formed. He used toluene as a preservative, and no coloration was produced without the use of ferrous sulphate.

Gortner<sup>16</sup> noted two varieties of tyrosinase in the meal worm, *Tenebrio molitor*, one water-soluble, the other water-insoluble, the latter in greater amount. The enzyme is uniformly distributed, but the color pattern in the potato beetle, for instance, was thought to be due to a localized secretion of chromogen. In his analyses published in 1911 Gortner<sup>17</sup> found that melanin on hydrolysis yielded tyrosine, lysine, arginine and 10 per cent "melanohumin," a brownish-black residue. Hence he regarded melanin as essentially of protein nature, a conclusion supported by the recent immunologic studies of Adant<sup>18</sup> at the University of Louvain. The inability of chemists to isolate the basic pigmented factor has led to the use of the term melanins, suggesting that their number may be legion. Gortner<sup>17</sup> stated that all melanoproteins are melanin, but not all melanins are melanoproteins. Some are acid-soluble. Those of keratin are insoluble in alkali until some decomposition has set in. The melanoproteins stain keratin diffusely as in auburn hair. Gortner further found that *m-di*-hydroxyphenols (orcinol, resorcinol and phloroglucinol) inhibit the oxidation of tyrosine by tyrosinase, yet do not destroy the tryrosinase, nor are they themselves oxidized in preference, their presence being sufficient to inhibit the reaction. While no similar chemical has as yet been isolated from a dominant white organism, this test tube experiment is at least suggestive of the mechanism that may be involved. Dominant whites, he holds, are due to the presence of anti-oxidase, while regressive whites have not the power either to form pigments or to inhibit their formation.

The old idea that melanin is derived from hemoglobin no longer has adherents. Mierowsky's<sup>19</sup> studies with incubated skin were strongly against this hypothesis. He found also a pyronine-red substance in

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13. Roques, X.: Compt. rend. Acad. d. sc. **149**:418, 1909.

14. Phisalix, M. C.: Compt, rend. Soc. de biol. **57**:17, 1905.

15. Durham, F. M.: Proc. Roy. Soc., London **74**:310, 1904.

16. Gortner, R. A.: J. Biol. Chem. **10**:89, 1911.

17. Gortner, R. A.: Am. Naturalist **45**:743, 1911; J. Biol. Chem. **10**:113, 1911.

18. Adant, M.: Arch. internat. de méd. expér. **7**:698, 1932.

19. Mierowsky, E.: Ztschr. f. Path. **2**:438, 1909.

cell nuclei, and this substance Dyson <sup>20</sup> thought is the chromatinprotein part of the melanin granule.

Piettre <sup>21</sup> hydrolyzed animal melanin with 25 per cent sulphuric acid and obtained amorphous amino-acids, tyrosine, leucine and a melanotic pigment. This pigment was insoluble in acids but very soluble in alkali. Melanin easily loses ammonia in alkaline solution.

Young, <sup>22</sup> in studying the pigment in the skin of an Australian black, extracted it in warm 5 per cent potassium hydroxide. The filtrates on cooling deposited much fat, which was removed. The final clear filtrate was acidified with hydrochloric acid; the pigment was precipitated as a dark brown powder, redissolved repeatedly in 5 per cent potassium hydroxide, filtered and reprecipitated and thoroughly washed with distilled water, dried, washed with alcohol, ether and carbon disulphide and dried in a vacuum over sulphuric acid. This powder was quite hygroscopic. Analyses showed it to contain carbon, hydrogen, nitrogen and sulphur and a trace of iron. When tested in solution spectroscopically, it was found that all rays in the violet, blue and part of the green were absorbed to a wavelength of about 515 microns.

Fürth and Schneider <sup>11</sup> regarded melanin as depending on the action of tyrosinase in conjunction with autolytic enzymes, the latter being freed from the chromogen groups of the protein molecule and then oxidized by the tyrosinase, undergoing condensation and taking up sulphur and iron, and possibly other elements, the entire complex forming the melanin. Sulphur and iron were considered as not indispensable constituents of the melanin molecule.

While there is a lack of unanimity regarding the constancy of sulphur in the melanin molecule, so many workers have stated definitely that melanin contains sulphur that it seems apropos to delve more deeply into the ubiquity of sulphur in human cells. The recent attention given to the sulphydril compounds has necessitated research with the sulphur-containing amino-acids, the most important of which is cystine. Ropshaw <sup>23</sup> regarded melanogenesis as nature's microcolor reaction in which the cysteine-cystine complex acts as an indicator, revealing a phase of nuclear metabolism, viz., the cleavage of protamine and its diffusion into the cytoplasm. Cysteine, he stated, is present in the cytoplasm within the more complex body, the tripeptide glutathione, and protamine is present in nucleoprotein (chromatin). The cleavage caused by cell enzymes results in these two compounds being freed and brought in contact. In vitro, a black precipitate is formed when protamine and

20. Dyson, W.: *J. Path. & Bact.* **15**:298, 1910.

21. Piettre, M.: *Compt. rend. Acad. d. sc.* **153**:782, 1911.

22. Young, W. J.: *Biochem. J.* **8**:460, 1914.

23. Ropshaw, H. J.: *Am. J. Physiol.* **103**:535, 1933.

cystine are brought together. In time the color disappears. Possibly this may indicate the mechanism whereby melanin reverts to a colorless form. In the skin, incidentally, the sulphhydryls are limited to the epithelial layer. Sulphur is also abundant in the appendages of the skin, such as the nails.

Hartwell<sup>24</sup> found that the more tyrosinase and tryptophan in the diet of rats the darker their coats became. Lignac,<sup>25</sup> on the other hand, showed that boiled tissues develop pigment under a quartz light and suggested that it was formed by a polymerization of quinidines. Also injections of pyrrole produced melanuria; therefore, melanin might be a simple cyclic complex of the pyrrole nucleus.

In most discussions on melanin, brown or blackish colors have been assumed, too uncritically it seems to me, to be melanin, or "a melanin," of "melanins." It is to be hoped that the term melanin can soon be used correctly only in the singular and with a definite idea as to a fundamental pigmented factor which, however, may have the ability to link itself with other substances, such as fat, lipoid, sulphur or metals, without altering its essential character.

Attempts at producing melanin in vitro have naturally had as their first aim the production of a black or brownish-black pigment. The aromatic compounds of the protein molecule, such as tyrosine, phenylalanine and tryptophan, readily condense with the elimination of water to form colored substances. Artificial melanin or melanoid substance can be formed by heating protein with hydrochloric acid. On decomposition with fused potassium hydroxide, it yields skatole, indole and pyrrole derivatives, derived from tyrosine and tryptophan. Bloch<sup>26</sup> has noted that the artificial melanin from tyrosine varies in its nitrogen content from 4.3 to 8.5 per cent. It seems probable that melanoid and melanin are formed from chromogen groups of the protein molecule through a process of condensation, elimination of water and the taking up of oxygen.

Brahn<sup>27</sup> derived a melanoidin from keratin and believed it very similar in composition to melanin to which he gave the structure 3, 4 *di*-hydroxyphenylcysteine.

In the ink sac of the cuttle fish and the meal worm and in plants producing Japanese lacquer there have been found oxidizing enzymes that can produce black pigment by their action on tyrosine solutions.

Neuberg,<sup>28</sup> with extracts of a melanosarcoma of the suprarenal gland, produced pigment from epinephrine and *B*-oxyphenylethylamine,

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24. Hartwell, G. A.: *Biochem. J.* **17**:547, 1923.

25. Lignac, G. O. E.: *Virchows Arch. f. path. Anat.* **240**:383, 1923.

26. Bloch, B.: *Am. J. M. Sc.* **177**:609, 1929.

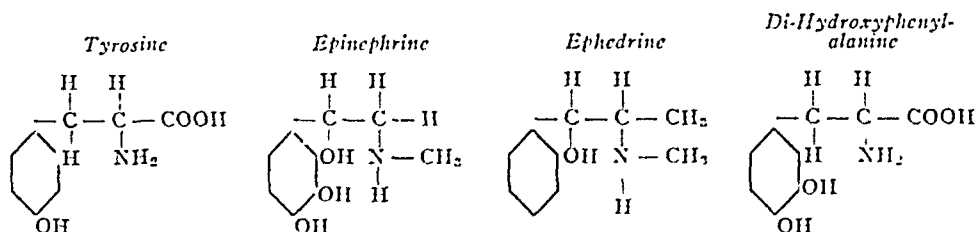
27. Brahn, B.: *Virchows Arch. f. path. Anat.* **227**:137, 1920.

28. Neuberg, C.: *Ztschr. f. Krebsforsch.* **8**:195, 1909.

but not from tyrosine. The ink sac of the squid contains an enzyme forming a pigment from epinephrine, also apparently through oxidation and condensation.

Winternitz<sup>29</sup> found in the uvea of the hog and the black subcutaneous tissue of the horse an enzyme causing darkening of tyrosine. These enzymes might hence be responsible for the production of melanin in animal tissues by causing oxidative changes in the chromogen groups of the protein molecule that are liberated by autolysis (Wells<sup>30</sup>).

It might be of advantage to indicate the structural relationships of tyrosine, epinephrine, ephedrine and *di*-hydroxyphenylalanine.



#### THE REACTION TO DI-HYDROXYPHENYLALANINE

Among the modern students of pigmentation the name of the late Bruno Bloch<sup>31</sup> of Zurich occupies a leading place. His studies have thrown much light on the origin of melanin and have been of great aid in the understanding of melanotic tumors, filling in certain gaps in the Soldan-Masson theory of the nervous origin of melanomas. In trying to account for the diffuse melanosis in Addison's disease, he reasoned that here was a condition in which the melanin deposits were associated with a lesion in an organ, the suprarenal gland, the main product of which is a pyrocatechin derivative, epinephrine.

He then used the cyclic amino-acid most closely related to epinephrine, *di*-hydroxyphenylalanine. Frozen sections of fresh skin were placed in 1 per cent *di*-hydroxyphenylalanine for from two to four hours at room temperature. In the cells which he regarded as those capable of forming melanin, the melanoblasts, the *di*-hydroxyphenylalanine was converted into a dark insoluble pigment, or *di*-hydroxyphenylalanine melanin. In other words, these cells contained an oxidase, or *di*-hydroxyphenylalanine oxidase. The cells did not develop melanin when exposed to pyrrole, tryptophan, tyrosine or epinephrine. The reaction appears to be definitely of a fermentative nature and is positive

29. Winternitz, R.: Arch. f. Dermat. u. Syph. **126**:252, 1918.

30. Wells, H. G.: (a). Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 528; (b) p. 533.

31. Bloch, B., and Ryhiner, P.: Ztschr. f. d. ges. exper. Med. **5**:179, 1917.

only in the cytoplasm (an argument against the nuclear origin of melanin).

The reaction to *di*-hydroxyphenylalanine is an indicator of the presence of a pigment-forming oxidase, but *di*-hydroxyphenylalanine is not necessarily the mother substance of natural melanin. It indicates, however, that pyrocatechin derivatives may play a rôle as substrates in the formation of the natural pigment.

The only physiologic pyrocatechin derivative is epinephrine. Pyrocatechin derivatives are occasionally found in the urine of patients with general melanomatosis, melanemia and melanuria; hence Bloch thinks that it is logical to believe that the derivatives come from the mother substance of melanin, becoming free through the disintegration of tumor cells, finally entering the blood and being excreted by the kidneys. An important exception to the so-called specificity of *di*-hydroxyphenylalanine in indicating melanoblasts is the polymorphonuclear leukocyte the oxidase of which forms a black pigment with *di*-hydroxyphenylalanine. The enzyme concerned is probably a polyphenolase and is capable of oxidizing many phenols to colored products.

The reaction to *di*-hydroxyphenylalanine is of importance in the understanding of the chemistry of melanin. Its significance in cellular studies will not be discussed in extenso here. Bloch's main contribution basically was in showing that in the formation of melanin, ferment processes play an important rôle. Lemmel,<sup>32</sup> on the other hand, questioned the enzymatic nature of the reaction since he found the reaction to *di*-hydroxyphenylalanine more active in boiled than in fresh tissues, but it is difficult to reconcile this with the fact that *di*-hydroxyphenylalanine oxidase dissolves quickly in water and is dispersed (Laidlaw<sup>33</sup>).

*Di*-hydroxyphenylalanine, as can be seen from its molecular structure, is an amino-acid similar in some respects to epinephrine. It occurs in the Georgia velvet bean (*Stizolobium deeringianum*) and in the seeds of *Vicia faba* (Miller<sup>34</sup>). It reacts more or less like epinephrine but does not have the highly marked physiologic properties, although distinctly toxic. It is apparently the chromogen involved in certain brown and black animal pigmentations, such as butterfly wings (*Vanessa antiopa*, Hasebroek<sup>35</sup>).

Bloch and Schaaf<sup>36</sup> cited additional evidence for the specificity of *di*-hydroxyphenylalanine oxidase. The reaction to *di*-hydroxyphenylalanine of human skin is given only by the levorotatory *di*-hydroxy-

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32. Lemmel, A.: *Centralbl. f. allg. Path. u. path. Anat.* **32**:89, 1921.

33. Laidlaw, G. F.: *Am. J. Path.* **8**:41, 1932.

34. Miller, E. R.: *J. Biol. Chem.* **44**:481, 1920.

35. Hasebroek, K., cited by Gortner, R. A.: *Outlines of Chemistry*, New York, John Wiley & Sons, Inc., 1928, p. 452.

36. Bloch, B., and Schaaf, F.: *Klin. Wchnschr.* **11**:10, 1932.

phenylalanine and not by the dextro form. Tyrosinase acts on both forms. The levorotatory compound is the natural form and is probably the propigment (Peck, Sobotka and Kahn <sup>37</sup>).

Spencer <sup>38</sup> has studied the chemical relationships of melanin, showing that protein derivatives like tyrosine, which contain the groups pyrrole, skatole or indole, are capable of being changed into melanin when acted on by certain enzymes which are found to occur frequently in plants and in the blood of insects and crustacea, and to their action the darkening of the blood of the animals on exposure to the air has been attributed (Alsberg <sup>39</sup>).

The dark color fungi assume when dying is dependent on an oxidizing ferment, for tyrosine, when mixed with an emulsion of fungi, turns black. The ferment obtained from the pupae of butterflies, when treated with tyrosine, forms a black pigment identical with melanin (McDonagh <sup>40</sup>). In 1887 Thormachlen <sup>41</sup> discovered in the urine of patients suffering from melanotic cancer of the liver a substance which darkened on exposure to air, but was intensified by treatment with oxidizing agents. This discovery suggested that the mother substance of melanin might be a colorless chromogen, melanogen.

The subcutaneous injection of melanin into guinea-pigs and rabbits is followed by the appearance in the urine of a substance which turns dark brown after the urine has stood for several days (Helman <sup>3</sup>). The pigment is said to be changed by the liver to the colorless form and by oxidation in the urine again assumes its pigmented character. Oxidizing agents will hasten the reaction. Helman has laid down three criteria for the presence of melanogen in the urine: First, the addition of ferric chloride must cause the development of a black precipitate. Second, this precipitate must dissolve in a solution of sodium carbonate. Third, mineral acids must also precipitate a black or brownish-black powder. There may be confusion in cases of chronic phenol intoxication and extreme indicanuria (Feigl and Querner <sup>42</sup>).

Eppinger <sup>43</sup> noted that the feeding of tryptophan to patients with melanuria increases the excretion of melanin. He found that in patients with melanoma the power of the body to destroy the pyrrole ring is reduced, with the result that it undergoes reduction and methylation with sulphuric acid to form a sulphate of methyl-pyrrolidine-hydroxycarbonic acid,  $\text{CH}_3\text{-C}_5\text{H}_9\text{N}_2\text{O}_4$ .

37. Peck, S. M.; Sobotka, H., and Kahn, J.: *Klin. Wchnschr.* **11**:14, 1932.

38. Spencer, W. G.: *Brit. M. J.* **2**:907, 1923.

39. Alsberg, C. L.: *J. M. Research* **16**:117, 1907.

40. McDonagh, J. E. R.: *Brit. J. Dermat.* **22**:316, 1910.

41. Thormachlen, J.: *Virchows Arch. f. path. Anat.* **108**:317, 1887.

42. Feigl, J., and Querner, E.: *Deutsches Arch. f. klin. Med.* **123**:107, 1917.

43. Eppinger, H.: *Biochem. Ztschr.* **28**:18, 1910.



Abderhalden<sup>44</sup> found a substance rich in tryptophan in the urine of a patient with melanuria. Primavera<sup>45</sup> noted in the urine of a patient with melanoma free tyrosine which fluctuated in amount with the pigment.

The pigment in Addison's disease has been assumed to be melanin. The circumstantial evidence is in favor of this assumption. Eiselt<sup>46</sup> isolated from the urine in Addison's disease a pigment rich in sulphur which he considered to be identical with melanogen. Jacobsen and Klinck,<sup>47</sup> utilizing the ability of melanin to reduce silver nitrate, found in the urine of two patients with Addison's disease renal casts containing a brownish and black pigment which was very argyrophilic and which they concluded was melanin. In the kidneys of patients with Addison's disease they also found much similar pigment in renal casts in situ and in the cells of the cortical and medullary tubules. The distribution of this pigment was the same as that in the kidneys of mice bearing large melanomas and in human melanomatosis.

Melanin, when injected parenterally, becomes decolorized by the reducing action of the tissues which blacken when oxidized. Quattini<sup>48</sup> injected pyrrole, indole and skatole subcutaneously into rabbits and found that in from nine to eighteen days the skin became darkened, and there was increased growth of pigmented hair. These observations suggest that a mother substance belonging to the indole group may be acted on by an enzyme produced in the epidermal cells, with the production of a melanin pigment (Dawson<sup>49</sup>). When melanin from melanotic tumors is treated with strong alkali, it yields indole and skatole and various volatile fatty acids, and under dry heat melanin gives off pyrrole.

Melanosis coli or melanotic pigmentation of the colon is of great interest in the study of the formation of melanin. Beneath an intact epithelial covering the stroma of the mucosa of colon, and often too of the appendix, is crowded with cells containing a brownish-black pigment which in the gross gives a grayish-black color to the lining of the intestine. The pigment looks like melanin and has affinity for silver salts. The pigment-bearing cells are negative to *di*-hydroxyphenylalanine, indicating that they do not actually form the pigment and hence must be phagocytes which have engulfed it. The condition apparently occurs only in very constipated people or when there is a chronic intestinal obstruction of mechanical origin.

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44. Abderhalden, E.: *Ztschr. f. physiol. Chem.* **78**:159, 1912.

45. Primavera, cited by Wells, H. G.: *Chemical Pathology*, Philadelphia, W. B. Saunders Company, 1925, p. 531.

46. Eiselt, R.: *Ztschr. f. klin. Med.* **69**:393, 1910.

47. Jacobsen, V. C., and Klinck, G. H., Jr.: *Arch. Path.* **17**:141, 1934.

48. Quattini, M., cited by Spencer.<sup>38</sup>

49. Dawson, J. W.: *Edinburgh M. J.* **33**:657, 1925.

Recently, in discussing the mobilization and excretion of melanin, Jacobsen and Klinck<sup>47</sup> suggested that melanosis coli is possibly evidence that the intestinal tract is one of the main routes for the escape of melanin after its discharge from effete body cells normally containing it. They had shown also that the kidneys are another important channel for the escape of surplus melanin. In melanosis coli there are complicating factors which in view of what is known of the composition of the melanin molecule make it necessary to consider the possibility of synthesis of melanin in the intestinal canal, the amino-acids and tyrosinase probably being present, and the whole being more of an *in vitro* than an *in vivo* process.

Ludwig Pick,<sup>50</sup> however, expressed the belief that the aromatic protein degradation products are absorbed from the large intestine and converted into melanin within the connective tissue cells by a ferment resembling tyrosinase. The melanin in melanosis coli varies somewhat in its reactions, at times reacting slowly to silver nitrate and being bleached by hydrogen dioxide with more difficulty than is the pigment of the skin (Lignac<sup>25</sup>).

An interesting but rare disease which throws some light on the chemical relationships of melanin is ochronosis. In 1866 Virchow first described it with its pigmentation of the cartilages and ascribed the pigment to hemoglobin derivatives. Von Hansemann<sup>51</sup> noted melanuria with the condition, and Hecker and Wolf<sup>52</sup> confirmed this observation. Albrecht<sup>53</sup> suggested a relationship to alkaptonuria, having found homogentisic acid in the urine. In 1906 Ludwig Pick<sup>54</sup> studied the condition and summarized the matter as follows:

Ochronosis is a definite form of melanotic pigmentation, the pigment of ochronosis being in most cases closely related to melanin. The pigment or its chromogen, circulating freely in the blood, is imbibed not only by cartilage, but also by loose connective tissue, voluntary and involuntary muscle cells and epithelial cells, without any obvious decrease in vitality of these cells. Degenerated tissues show the greatest amount of pigmentation. The diffuse pigment may become granular after a time. It is iron-free, but under certain circumstances may contain fat. The melanin arises from the aromatic nucleus of the protein molecule (tyrosine, phenylalanine), and the related hydroxylized products, under the influence of tyrosinase.

In some cases the constant absorption of minute quantities of phenol from surgical dressings has apparently caused the condition. Besides this formation of pigment from such exogenous aromatic substances,

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50. Pick, L.: *Berl. klin. Wchnschr.* **43**:478, 1906.

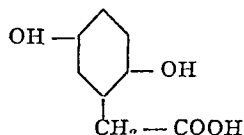
51. von Hansemann, D.: *Berl. klin. Wchnschr.* **29**:660, 1892.

52. Hecker, A., and Wolf, H., cited by Wells, H. G.: *Chemical Pathology*, Philadelphia, W. B. Saunders Company, 1925, p. 532.

53. Albrecht, H.: *Ztschr. f. Heilk.* **23**:366, 1902.

54. Pick, L.: *Berl. klin. Wchnschr.* **48**:840, 1911.

it is probable that in alkaptonuria the endogenous aromatic substance, homogentisic acid, may be converted into pigment by tyrosinase. In many cases of ochronosis the pigment or a precursor may be excreted in the urine, which then undergoes spontaneous darkening when exposed to the air. The kidneys may also become pigmented, and granular masses of pigment may be present in the renal tubules. The evidence, as Wells<sup>30b</sup> stated, is quite conclusive of the origin of ochronotic pigment from aromatic radicals, whether these radicals are converted into true melanin or not. The structure of homogentisic acid is:



A type of ochronosis which affects the bones rather than the cartilages in the lower animals also occurs. Schmey<sup>55</sup> has called this condition osteohemochromatosis, and others have called it osteohematophyria. If it is comparable with a pigmentation of bones which occurs in fish, the pigment is not of blood origin but is true melanin in all probability. Harper<sup>56</sup> observed extensive melanism in the skeleton of *Raia maculata*, every portion of the skeleton containing a deposit of melanin pigment. The deposit was heaviest in the main cartilages. The pigment was extracted with methyl alcohol acidified with hydrochloric acid. This solution after filtration showed no absorption bands, which excluded all compounds of the porphyrin class and, in particular, uroporphyrin. It was bleached by hydrogen dioxide or nitric acid, indicating that the pigment probably belongs to the melanin type. Gross<sup>57</sup> has produced ochronosis experimentally by the administration of homogentisic acid, but numerous cases are on record of alkaptonuria without ochronosis. Some profound metabolic disorder involving the catabolism of tyrosine and phenylalanine seems at fault, and usually it is of hereditary nature. The presence of homogentisic acid in the urine should not be taken necessarily as an indication of a pathologic condition, since an increased consumption of proteins containing tyrosine and phenylalanine will increase the amount of homogentisic acid which is excreted.

In spite of much contradictory research on the structure of the melanin molecule, there appears to be general agreement on certain basic facts. When tyrosinase acts on tyrosine, a series of changes in color occurs, most rapidly at  $p_H$  6 and more slowly between  $p_H$  6 and 8.

55. Schmey, M.: Frankfurt. Ztschr. f. Path. **12**:218, 1913.

56. Harper, W. F.: J. Path. & Bact. **35**:865, 1932.

57. Gross, O.: Deutsches Arch. f. klin. Med. **128**:249, 1919.

At first a red color develops which passes into a brown or black. The red color, according to Laurens,<sup>58</sup> becomes decolorized, forming an easily oxidizable substance. The red color is the primary result of the action of the tyrosinase, the subsequent changes being purely oxidations. It is probable that the first product of the action of tyrosinase on tyrosine is the formation of *di*-hydroxyphenylalanine. On oxidation this becomes the corresponding quinone, which in turn is converted into 5:6 *di*-hydroxy-*di*-hydroindol-2-carboxylic acid. This by oxidation becomes the corresponding quinone, which is probably the red substance, the first visible product of the enzyme action. When the red substance is decolorized it gives rise to 5:6 *di*-hydroxyindol or its 2-carboxylic acid, both probably easily oxidized in the air and one of which must be the colorless melanogen.

Since the only physiologic pyrocatechin derivative is epinephrine, and since pyrocatechin derivatives play their most conspicuous rôle in melanomatosis, the coincidence carries weight as indicating that pyrocatechin derivatives come from the mother substances of melanin, which are formed in great amount, are freed through break-up of tumor cells, enter the blood and are eliminated through the kidneys and probably also through the intestinal tract.

Melanin has some of the characteristics of a true protein or colloid and is classed by Gortner as a conjugated protein, subgroup chromoprotein or melanoprotein. The recent studies of Adant seem to indicate that melanin has also one of the biologic properties of proteins; i. e., it is antigenic. A knowledge of this fact opens a field of research which may result in important discoveries concerning normal and pathologic pigmentation. An appreciation of the normal variations in the threshold of secretion and excretion of melanin in conjunction with an accurate estimate of its antigenic potency may lead to the development of a serologic method for the determination of familial and racial characters based on potential or actual melanogenesis.

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58. Laurens, H. L.: *Physiological Effects of Radiant Energy*, New York, The Chemical Catalog Company, Inc., 1933, p. 134.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—According to *Science*, G. P. Wright has been appointed to the Sir William Dunn chair of pathology tenable at Guy's Hospital Medical School. Since 1931 he has been assistant lecturer in morbid anatomy and curator of the Museum at University College Hospital Medical School and also pathologist of the hospital.

Bradley M. Patten, associate professor of histology and embryology in Western Reserve University has resigned to become assistant director for medical sciences for Rockefeller Foundation.

Albin Haberdia, professor of legal medicine in the University of Vienna and author of a widely used textbook of legal medicine, has died at the age of 66, after a long illness.

**Society News.**—At a conference held in Washington on Feb. 5 and 6, 1934, under the auspices of the National Research Council there was organized the American Academy of Tropical Medicine with Theobald Smith as president, Charles F. Craig as vice-president, W. W. Cort as treasurer, E. B. McKinley as secretary, and a council of five.

George B. Hassin has been elected president, and Armando Ferraro secretary, of the American Association of Neuropathologists.

The German Pathological Society will hold its next meeting in Rostock May 23 to 25, 1934.

**Bequests.**—According to *Science News-Letter*, the University of Wisconsin has received a bequest from the late Miss Jean Bowman to found a cancer clinic and research center.

**Grants-in-Aid.**—The Committee on Grants-in-Aid of the National Research Council announces that it has received a new grant for the year 1934. Formal blanks for use in applying for a grant will be furnished on request to the committee, 2101 Constitution Avenue, Washington, D. C. The Committee on Scientific Research of the American Medical Association has been given a new appropriation by the trustees of the Association. Application blanks will be sent on request to 535 North Dearborn Street, Chicago.

## Obituaries

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MAJOR PAUL EDGAR McNABB. M.C., U.S.A.

1887-1934

Major Paul Edgar McNabb died on Feb. 24, 1934, from a rapidly progressive hypertensive disease and cerebral hemorrhage. He was born in Sevier County, Tenn., on May 23, 1887, and at the age of 5 years moved with his parents to Knoxville, Tenn., where he spent his



MAJOR PAUL EDGAR McNABB. M.C., U.S.A.

1887-1934

boyhood. He graduated in medicine at the University of Pennsylvania in 1912, and then practiced medicine for several years with his father, Dr. Charles P. McNabb, a distinguished physician in Knoxville.

Major McNabb was an honor graduate of the Army Medical School in 1917, and served in France during the World War. He graduated from the advanced course, Army Medical School, in 1921, and was assigned to the laboratory service of the medical department, where he served with distinction in many difficult assignments, including the

Second Corps Area Laboratory, New York; Board of Health Laboratory, Ancon, Canal Zone; Walter Reed General Hospital, and the Army Medical School. During this service he came to be regarded as one of the outstanding pathologists in the Army Medical Corps. He was curator of the Army Medical Museum from February, 1931, to January, 1933, and was president of the U. S. Army Medical Department Research Board in Manila, P. I., at the time he was stricken with the illness which forced him to return to the United States.

Always interested in research, he published articles on postmeasles pneumonia, quinine prophylaxis in army troops in the Canal Zone, coronary sclerosis in angina pectoris and the presence and significance of albuminuria in the personnel of a citizens' military training camp. In collaboration with others, he published articles on hemochromatosis and congenital heart block.

Major McNabb was a fellow of the American Medical Association and of the American College of Physicians and was a member of the American Association of Pathologists and Bacteriologists, the International Association of Medical Museums and the National Board of Medical Examiners representing the U. S. Army Medical Corps. He was also an honorary life member of the Knox County Medical Society.

An indefatigable worker for the interests of the laboratory service, Major McNabb was keenly interested in the welfare and advancement of its personnel, especially of the junior members of that service. Possessed with professional ability, charming personality and more than ordinary tact, he quickly obtained the friendship and respect of those who were privileged to be associated with him. His untimely death during the years of greatest productivity is a distinct loss to the Medical Corps.

In 1913, he married Miss Therese Franz of Knoxville, who with their daughter, Jane Gordon McNabb, aged 17, survives him.

V. H. CORNELL.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

COMPLETE HEART-BLOCK IN HYPERTHYROIDISM FOLLOWING ACUTE INFECTIONS. AUSTIN C. DAVIS and HARRY L. SMITH, *Am. Heart J.* 9:81, 1933.

Six cases of exophthalmic goiter in which complete auriculoventricular dissociation occurred were observed. Three of the patients had had acute tonsillitis shortly preceding the arrhythmia, and one patient had had scarlet fever. In the one fatal case, there was an inflammatory lesion in the region of the bundle of His, in which were gram-positive cocci. In the two cases in which the heart block was not associated with acute infection, the block may have been precipitated by the effect of digitalis on a previously injured auriculoventricular bundle. In those cases in which acute infection was present, the evidence indicates that the hyperthyroidism predisposed the patient to the development of acute infection and also to secondary myocardial involvement.

FROM AUTHORS' SUMMARY.

LYMPHATIC LEUKEMIA FOLLOWING BENZOL POISONING. ERNEST H. FALCONER, *Am. J. M. Sc.* 186:353, 1933.

A case of benzene poisoning is presented in which the patient recovered for a time, showing a lower level of blood cell production, which gradually passed into true lymphatic leukemia (verified at autopsy) with a fatal termination. Instances are drawn from the literature suggesting that benzene, like other agents destructive of hemopoietic tissue, may upset the balance of an orderly production and destruction of blood cells, bringing about a tissue environment favorable to the production of lymphatic leukemia. Attention is directed to the direct division of the nucleus in many lymphatic cells in the blood stream and in lymphatic tissue cells in lymph nodes and bone marrow.

AUTHOR'S SUMMARY.

POSTERIOR PITUITARY ACTIVITY FROM AN ANATOMICAL STANDPOINT. HARVEY CUSHING, *Am. J. Path.* 9:539, 1933.

In all the recent attention to the pituitary body and its functions, the posterior lobe has been much neglected because of the greater temporary interest in the hormones of the pars anterior and the newly discovered activities of the interbrain. It nevertheless has an active principle or principles capable among other properties (1) of raising the blood pressure, (2) of contracting smooth muscle, (3) of causing hyperglycemia, (4) of expanding cutaneous melanophores and (5) of diminishing renal secretion. Its secretory product is unmistakably derived from the investing pars intermedia, the cells of which become basophilic when ripened. Doubtless, under nervous impulses from hypothalamic nuclei, these cells, by a form of holocrine secretion, are cast off, invade the pars nervosa and become transformed into "hyaline bodies," which, as Herring first showed in lower animals, make their way through the loose neural spaces of the pars nervosa in the direction of the infundibular cavity. They may, in favorable preparations from the cat and dog, even be seen to extrude themselves into the ventricle between the ependymal cells. In certain conditions of disease characterized notably by hypertension, but also by other symptoms suggestive of known physiologic effects of posterior lobe extracts, the normal cellular activity of the pars intermedia becomes greatly exaggerated. This is shown by a marked hyperplasia of the basophilic elements that penetrate far into the lobe. Under these circumstances, not only is the visible secretory product (hyalin) greatly increased in amount over what is customarily seen in supposedly normal glands, but the cuticular ependyma of the lower third ventricle



has a highly broken up appearance. An extreme degree of cellular hyperactivation of the posterior lobe was first observed in the pituitary body in a fatal case of what is known as pituitary basophilism associated with a basophilic adenoma of the pars distalis. Since then, a similar condition has also been found in cases of eclampsia and of so-called essential hypertension. In both of these states, there has been found in the blood stream a substance indistinguishable in its effects from posterior lobe extract, but not detectable in the blood under normal conditions. The conclusion is drawn that the infiltrative basophilia of the pars nervosa is an expression of functional hyperactivation of the posterior lobe and may be taken to represent the pathologic basis of these hypertensive disorders. What particular form of neurohumoral stimulus primarily incites the neurohypophyseal basophilia remains to be determined.

AUTHOR'S SUMMARY.

PHOSPHORUS AND ALCOHOLIC CIRRHOSIS. F. B. MALLORY, *Am. J. Path.* **9**:557, 1933.

Very chronic poisoning with minute daily doses of yellow phosphorus produces, in five months or more, cirrhosis of the liver in rabbits and in guinea-pigs. The hepatic cells undergo degenerative changes similar to those found in alcoholic cirrhosis. They contain granules and hyaline networks (reticula) that are acidophilic and give the reaction for phosphates by the Macallum and Fiske-Subbarow tests. By a special method, it is possible to demonstrate free phosphorus when cast iron and tin plate are dissolved in dilute hydrochloric acid. Phosphorus may get into alcohol and liquors through acid erosion of iron and tin plate vessels and containers and thus contaminate them and be the cause of alcoholic cirrhosis.

AUTHOR'S SUMMARY.

ERYTHROLEUCOBLASTOSIS IN THE NEWBORN. R. C. WANSTROM, *Am. J. Path.* **9**:623, 1933.

A case of fetal erythroleukoblastosis with ascites is reported, in which microscopic study revealed certain unusual features. In addition to marked hematopoiesis in the liver, spleen, kidneys, bone marrow, lymph nodes, thymus, diaphragm, pharynx, small intestine and pancreas, similar foci were found in the chorionic villi. In the lesser bile ducts and in some renal tubules numerous giant cells were found, apparently resulting from alterative changes in damaged epithelium. The generic relationship and probable intrinsic constitutional etiology of congenital hydrops, icterus gravis and fetal erythroleukoblastosis of the new-born are pointed out and suggestions made as to future investigations along these lines.

AUTHOR'S SUMMARY.

EFFECT OF TESTICULAR EXTRACT ON EXPERIMENTAL TUBERCULOSIS IN RABBITS. T. T. WALKER and D. C. HOFFMAN, *Am. J. Path.* **9**:651, 1933.

When tubercle bacilli together with testicular extract are injected into the skin of rabbits, marked enhancement of the resulting lesions occurs. This enhancement takes place equally well with the human, bovine and avian strains. The practical application of this phenomenon in laboratory diagnosis is worthy of consideration.

AUTHORS' CONCLUSIONS.

BLOOD CHOLESTEROL IN EXPERIMENTAL THYROID DISEASE. J. J. WESTRA and M. M. KUNDE, *Am. J. Physiol.* **103**:1, 1933.

Artificially cretinized rabbits showed a cholesterol content of the blood greater than normal; after the feeding of desiccated thyroid, this was reduced—a phenomenon which does not always occur after a similar procedure in normal rabbits. No correlation could be established between these changes and the alterations of red cells and hemoglobin.

H. E. EGERS.

EXPERIMENTAL ALOPECIA. B. BARKER BEESON and W. J. PICKETT, Arch. Dermat. & Syph. **28**:53, 1933.

The sympathetic ganglion of the second cervical nerve was removed bilaterally in twenty-one cats. Seventeen of them survived, and six of the survivors showed postoperative alopecia, which appeared from the fourth to the fortieth day. The loss of hair attained its maximum in several weeks and then remained stationary for one or two weeks, after which the hair reappeared, becoming normal in from one to two and one-half months. Histologically atrophy and disappearance of hairs, hair follicles and sebaceous glands were noted. The subdermal connective tissue was thickened. These histologic changes are similar to those found in alopecia areata in human beings.

S. W. BECKER.

EFFECT ON THE REFLEXES OF THE CAROTID SINUS OF RAISING THE INTRACRANIAL PRESSURE. C. M. GUERNSEY, S. A. WEISMAN and F. H. SCOTT, Arch. Int. Med. **52**:306, 1933.

The rise of arterial pressure following an increase of intracranial pressure is, in dogs and rabbits, of central origin, as Cushing thought, and does not start in the carotid sinus. In the authors' experiments, the carotid sinus was always found to act as a depressor zone, tending to keep the pressure down even when the nerve centers needed blood. Following denervation of the sinus, increased intracranial pressure caused a more prompt and greater rise of arterial pressure. The differences before and after denervation were not great, and this was true in rabbits in which the influences of the aortic depressors also were removed.

AUTHORS' SUMMARY.

GALL BLADDER EPITHELIUM AS AN OSTEOGENIC STIMULUS AND THE PHYSIOLOGICAL DIFFERENTIATION OF CONNECTIVE TISSUE. C. B. HUGGINS and J. F. SAMMET, J. Exper. Med. **58**:393, 1933.

Evidence is presented that the proliferating epithelium of the gallbladder in the dog and guinea-pig is capable of stimulating the formation of bone in certain connective tissues such as the abdominal wall. Other areas of connective tissue such as the subepithelial connective tissue of the gallbladder and the urinary bladder do not share in this reaction and resist the osteogenic stimulus of the epithelium. In these circumstances, this formation of bone is biphasic. A difference between connective tissues morphologically identical can be proved physiologically by their response to the osteogenic stimulus of appropriate epithelia. Microliths of calcium carbonate occurred in the mucus of the occluded gallbladder in which there was transplanted connective tissue forming part of the wall.

AUTHORS' SUMMARY.

THE RACES THAT CONSTITUTE THE GROUP OF COMMON FIBROBLASTS. R. C. PARKER, J. Exper. Med. **58**:401, 1933.

Races of fibroblasts that are functionally distinct have been isolated from the various tissues and organs of a single chick embryo. Functionally distinct races of fibroblasts have also been isolated from corresponding parts of embryos of different ages. Under the conditions of the experiments, and for the particular races of fibroblasts that have been studied, it has not been possible to demonstrate a gradual decrease, or a gradual increase, in the rate of multiplication of fibroblasts obtained from corresponding parts of embryos of gradually increasing ages. Experiments made on strains of fibroblasts derived from the mesonephron and metanephron of a 16 day chick embryo have indicated that the rate of multiplication of these cells in a given medium reflects the physiologic state, at the moment of isolation, of the particular part of the embryo from which they are obtained. The rate of multiplication of a given race of fibroblasts in a particular medium does not serve, necessarily, as an index of the age of the individual from which

the race is derived. The functional differences that distinguish the various races of fibroblasts are permanent; they are retained indefinitely by the cells from passage to passage, despite such attempts as have thus far been made to change them.

AUTHORS' SUMMARY.

SYNERGIC HYPOPHYSEAL FACTOR AND PROLAN LIKE SUBSTANCES. H. M. EVANS, M. E. SIMPSON and P. R. AUSTIN, *J. Exper. Med.* **58**:545 and 561, 1933.

The hypophyseal substance—the synergic factor—which gives increased gonadotropic effects when combined with prolan, has been shown itself to possess a definite though slight gonadotropic activity. It produces transitory follicular enlargement within from twenty-four to forty-eight hours, which, however, regresses at once on cessation of treatment, so that by the end of the fourth day the ovary again approximates its infantile weight. The synergic principle indeed provokes only moderate ovarian development when administered in one hundred times the dose necessary to demonstrate the phenomenon of activation. By means of iso-electric precipitation or by means of tryptic and ereptic digestion, fractions containing the synergic principle were freed of many contaminants, in particular of the antagonistic factor. The synergic principle has been shown to be unaffected by digestion with trypsin or with trypsin followed by a short digestion with erepsin, but the action of pepsin inactivated the material. A preparation of the active substance has been obtained which is potent in a total dose of 27 micrograms.

The phenomenon of synergism has made possible the recognition of substances which the authors have called "prolan-like" in a wide variety of conditions. Indeed, it has been possible to demonstrate a prolan-like material in the urine of normal men. The method has shown a wide distribution of prolan-like substances in serums and urines of nonprimates without demonstrating, however, a significant difference in the reactions secured from pregnant, as contrasted with nonpregnant, states, or in males as contrasted with females. The phenomenon of synergism with the prolan of pregnancy can also be shown in the increased development of the seminal vesicles of immature male rats. However, such males cannot be advantageously employed in the detection of prolan-like gonadotropic substances occurring in the blood and urine in conditions other than pregnancy, for the prolan-like substances usually do not effect appreciable development of the seminal vesicles.

AUTHORS' SUMMARIES.

CONCENTRATION OF THE GONADOTROPIC HORMONE IN PREGNANT MARE'S SERUM. H. M. EVANS, E. L. GUSTUS and M. E. SIMPSON, *J. Exper. Med.* **58**:569, 1933.

The gonadotropic hormone of the blood of the pregnant mare has been greatly concentrated by adsorption on active aluminum hydroxide followed by elution. The preparations so obtained gave demonstrable gonadotropic effects within one hundred hours in 21 day old female mice following three subcutaneous injections of 0.001 mg. in 1 cc. of physiologic solution of sodium chloride. As is well known, other gonadotropic substances do not cause conspicuous development of the male gonads, but injections of comparatively large doses of these preparations into immature male rats caused marked development of the testes, which in ten days were trebled in weight. An astonishing increase in the weight of the seminal vesicles resulted, for these organs were approximately seventy-five times heavier than in controls.

AUTHORS' SUMMARY.

CULTIVATION OF MONOCYTES IN FLUID MEDIUM. L. E. BAKER, *J. Exper. Med.* **58**:575, 1933.

Monocytes from blood and from spleen have been cultivated in fluid medium in Carrel flasks for over two months. Diluted serum supplied all the essential

nutritive substances. Cultivation in fluid was made possible by adjusting the initial  $p_H$  of the fluid to 7.4, and not allowing it to fall below 7 or 6.8. The cells remained in good condition when the  $p_H$  was adjusted with lactic acid, hydrochloric acid or carbon dioxide. Adjustment with carbon dioxide was found to be more convenient and also more practical, since it does not destroy the buffer action of the medium. After two months of cultivation, the monocytes were in excellent condition and still proliferated actively. They gave every indication that indefinite multiplication could be maintained under the conditions of these experiments. It is hoped that this method of cultivation, with some modifications, will prove useful in studying the metabolism of these cells.

AUTHOR'S SUMMARY.

PRODUCTION IN DOGS OF CHRONIC BLACK TONGUE WITH ANEMIA. C. P. RHOADS and D. K. MILLER, *J. Exper. Med.* 58:585, 1933.

By the feeding of a particular diet, apparently lacking a substance closely associated with vitamin B<sub>2</sub> G, a chronic disease may be produced irregularly in dogs. The disease is characterized by atrophic glossitis, diarrhea, loss of weight and anemia. The disease can be prevented and relieved by materials rich in vitamin B<sub>2</sub> G.

AUTHORS' SUMMARY AND CONCLUSIONS.

METHODS AND EFFECTS OF INCREASING THE URINARY CONSTITUENTS IN THE BODY. F. W. HARTMAN, *J. Exper. Med.* 58:649, 1933.

Five methods for the production of slow, continued reabsorption of urine in the experimental animal are presented. The effects of this prolonged reabsorption of urinary constituents on the blood chemistry and tissues are shown with special reference to the kidney. Large and rapid increase of urinary constituents in the body is definitely destructive to the kidney. The degenerative changes found in the experimental animal in the protected pole of the kidney showing nephritis produced by roentgen rays and in man in the unscarred portions of the kidney showing chronic nephritis account for the progressive nature and final renal insufficiency of these conditions and are best ascribed to the high levels of excretory products in the body.

AUTHOR'S CONCLUSIONS.

THE RELATIVE RESPONSE TO BLOOD GAINS AND BLOOD LOSSES. M. DICK, *J. Exper. Med.* 58:707, 1933.

The effects of gradually increasing or diminishing the amount of circulating hemoglobin have been studied in rabbits. Contrary to expectation, it was found that when the pigment was increased by the injection of a small quantity of compatible blood every day during a number of weeks, the erythropoietic tissue did not lessen its activities. The percentage of hemoglobin mounted gradually yet considerably, even when as little as one hundredth of the amount of blood initially possessed by the animal was injected each day; and the figure it finally attained must in some instances, at least, have been expressive of a superabundance. To this superabundance the animal itself evidently contributed through its persisting erythropoietic activity. The results were very different when rabbits were bled daily, the same small amount of blood being lost that was injected into their fellows. The marrow became abnormally active, and this activity continued undiminished throughout the long period of the bleedings. The organism is evidently far more susceptible to loss than to gain of blood, a fact which is scarcely surprising when one considers that throughout its differentiation, it has had to cope with exigencies of the first sort only. Rabbits in which the hemoglobin is gradually increased by the injection of strange blood become so accustomed to the abundance of pigment that even a slight decrease in amount causes the erythropoietic tissue to become abnormally active to maintain the new status quo. Good reasons exist for referring the habituation thus manifested to readjustments in the functioning of the physiologic mechanisms which mediate between the demand for oxygen and the erythro-

poietic response. Too little recognition has been given to the rôle of these mechanisms in such a relation. No evidence was obtained of any effective readjustment to protect the erythropoietic tissue from the stimulus of daily small loss of blood.

AUTHOR'S SUMMARY.

FAILURE OF ADMINISTRATION OF IRON TO CAUSE HAEMOCHROMATOSIS. C. POLSON, *Brit. J. Exper. Path.* **14**:73, 1933.

The iron content of the liver of thirty-six adult rabbits was maintained at a high level for from one to four years. The terminal level in ten rabbits under experiment from three to four years ranged from 3.42 to 7.67, and averaged 5.19 per cent by dry weight. Cirrhosis of the liver and pancreatic damage were absent from all. It is therefore shown that excess of iron in the body over long periods caused neither cirrhosis of the liver nor hemochromatosis in rabbits, and it is unlikely that excess of iron is responsible for the hepatic and pancreatic lesions of human hemochromatosis.

AUTHOR'S SUMMARY.

THE CORTICAL LIPOID OF THE MOUSE SUPRARENAL AFTER UNILATERAL SUPRARENALECTOMY. RAYMOND WHITEHEAD, *Brit. J. Exper. Path.* **14**:149, 1933.

The relative amounts of sudanophil cortical lipid in both suprarenals of 137 unilaterally suprarenalectomized male white mice have been estimated. Right suprarenals removed at intervals of from three to fifty-six days after removal of the corresponding left glands showed a decrease in the amount of lipid. The proportion of right glands with decreased lipid rose to a maximum of 68 per cent at fourteen days after removal of the left glands, and gradually fell to 17 per cent at fifty-six days. Some right glands showed increased lipid from twenty-one to fifty-six days after removal of the left glands. Control laparotomies on twenty-four mice demonstrated that the lipid changes were not due to the surgical technic. The results indicate that the amount of lipid in depleted right glands in time attains or exceeds its original value. Histologic types of lipid change are described and illustrated. Lipoid changes as sources of error are discussed. No evidence of cortical hypertrophy in twenty-nine right glands removed fifty-six days after removal of the left was obtained.

AUTHOR'S SUMMARY.

RENAL LESIONS IN HYPERVITAMINOSIS D. J. GOUGH, J. B. DUGUID and D. R. DAVIES, *Brit. J. Exper. Path.* **14**:137, 1933.

The occurrence of calcification and parenchymatous degeneration ("nephrosis") in the rat's kidney in hypervitaminosis D was investigated. The two lesions, which frequently coexist, were found to be largely independent of one another. Calcification went parallel with a high renal excretion of calcium. Nephrosis could not be clearly correlated with any of the urinary disturbances recorded, but seemed to be in a large measure dependent on the administration of sodium biphosphate, U. S. P., with overdoses of vitamin D. The effects of large doses of the acid and alkaline orthophosphates, both with and without hypervitaminosis D (20,000 M.R.C. units of vitamin D substance daily), were compared. The urinary excretion of calcium and the deposition of calcium in the kidneys were found to be greatest when the alkaline phosphate was given, and especially when the vitamin D substance was added. The urinary excretion of phosphorus was greatest when the acid phosphate was given. The vitamin D substance had the effect of lowering it, both when the acid and when the alkaline salt was given. The amount of phosphorus found post mortem in the kidney was greatest when the vitamin D substance had been given with the acid diet, and it was in these circumstances that the most severe nephrosis developed. The large accumulation of phosphorus in the kidney in the nephrotic rats was thought to be a mark of retention of phosphates

caused by the renal disease. The urinary excretions of calcium and phosphorus appeared to show a reciprocal relationship to one another, a relative elevation of the one being accompanied by a corresponding lowering of the other.

AUTHORS' SUMMARY.

#### EXPERIMENTAL HYPERTENSION AND THE ARTERIAL LESIONS IN THE RABBIT.

M. KREMRR, SAMSON WRIGHT and R. W. SCARFF, Brit. J. Exper. Path. **14**:281, 1933.

The aortic and sinus nerves (buffer nerves) were extirpated in rabbits which then were allowed to survive for long periods. The changes in the blood pressure were recorded by the carotid loop method. Following unilateral denervation, there is an elevation of the blood pressure from the normal level (about 95 mm.) to about 115 mm. Following bilateral denervation, there is a persistent marked hypertension, the average blood pressure being 160 mm. Degenerative changes in the aorta and fibrosis of the heart may take place in a high proportion of cases following bilateral denervation. These changes are not observed in control animals or after unilateral operation. The mode of production of the lesions is uncertain, but they are not the invariable accompaniment of hypertension.

AUTHORS' SUMMARY.

### Pathologic Anatomy

#### EXFOLIATIVE DERMATOSIS AND MALIGNANT ERYTHRODERMIA. HAMILTON MONTGOMERY, Arch. Dermat. & Syph. **27**:253, 1933.

Seventy-three cases of exfoliative dermatitis and sixteen cases of generalized erythrodermia were studied. Exfoliative dermatitis following psoriasis presents a histologic picture similar to that of psoriasis, differing only in the more marked intercellular edema. Exfoliative dermatitis following eczema presents a varying degree of spongiosis. Exfoliative dermatitis due to arsenical poisoning presents a picture often indistinguishable from that of the same condition following eczema. In exfoliative dermatitis as a part of the picture of lymphoblastoma (Hodgkin's disease, leukemia, lymphosarcoma, mycosis fungoides) the amount of infiltrate in the cutis is larger, and the character differs, depending on the type of case. In mycosis fungoides the infiltration is limited to the upper portions of the dermis and is composed of a multiplicity of cell types, with pyknosis and karyorrhexis of the individual cells and a tendency toward clumping of many of the cells. The cells consist of eosinophils and polymorphonuclear leukocytes, fibroblasts, cells of the vessel walls and cells of the lymphocytic series. In Hodgkin's disease of the skin Dorothy Reed cells are found. In leukemia cutis there is dense infiltration with cells of the lymphocytic series or of the myelocytic series. Lymphosarcoma differs from lymphatic leukemia only in the numerous mitotic figures. In more than half of the cases of lymphoblastoma it is impossible definitely to specify which type of lymphoblastoma will eventually develop. It still seems advisable to attempt classification of the lymphoblastoma group as separate but related entities.

S. W. BECKER.

#### MYCOSIS FUNGOIDES. OLIVER S. ORMSBY and CLARK W. FINNERUD, Arch. Dermat. & Syph. **27**:631, 1933.

The case of a woman, aged 32, who had the prefungoid stage of mycosis fungoides which terminated fatally is reported. Sections from various regions of the skin showed the characteristic polymorphous cellular infiltrate simulating a granuloma. A periaortic abdominal lymph gland showed a delicate fibrillar network containing lymphocytes, eosinophilic polymorphonuclear leukocytes, a few lymph channels and a very few larger cells with a large nucleus, sparse chromatin and an abundance of eosinophilic cytoplasm. The spleen showed lobule-like proliferation about the central arteries. The authors believe that the changes in the spleen

and probably those in the lymph glands were similar and analogous to those in the skin, the pathologic cells in these internal structures representing a hyperplasia apparently derived from the lymphoid cells and the reticulum. They believe that the histologic changes are inflammatory or progressive and not due to an infiltration of neoplastic tissue. They also believe that the preponderance of clinical and histologic evidence demands the continued recognition of mycosis fungoides as a disease sui generis.

S. W. BECKER.

**PULMONARY EMBOLISM FROM ARSENICALS INJECTED INTRAVENOUSLY.** GEORGE C. SHIVERS, Arch. Dermat. & Syph. **27**:901, 1933.

A case is reported in which death resulted from a pulmonary embolism following the intravenous injection of 0.9 Gm. of neoarsphenamine as an initial dose. The author found that a low  $p_H$  caused precipitation of arsphenamine, neoarsphenamine, sulpharsphenamine and mersalyl in serum in vitro, and caused embolism when injected into rabbits. He found a surprisingly high number of low  $p_H$  values in neoarsphenamine obtained in the open market. The author advises testing neoarsphenamine by means of bromthymol blue, which has a sharp end-point at  $p_H$  7 when combined with neoarsphenamine.

S. W. BECKER.

**PRODUCTIVE-CICATRICIAL SYPHILITIC DISEASE OF THE PULMONARY ARTERY.** HOWARD T. KARSNER, Arch. Int. Med. **51**:367, 1933.

Productive-cicatrificial syphilitic disease of the pulmonary artery, i. e., of the Dohle-Heller type, is extremely uncommon, but occurs often enough to justify its consideration in clinical diagnosis. Eleven cases, anatomically proved by reasonably liberal interpretation, are now on record. Eight of these would be regarded as clearly established by the most exacting criteria. Gummatous types of lesion about equal the productive-cicatrificial type in incidence, but this ratio may be changed as additional cases are reported. The proportion of productive-cicatrificial to gummatous lesions is markedly higher in the aorta than in the pulmonary artery. All cases of productive-cicatrificial lesions in the main stem of the pulmonary artery show local or diffuse dilatation of the vessel. Associated disease of the smaller pulmonary arteries is by no means constant, but all cases have shown some degree of cardiac hypertrophy. Thrombosis is more frequently associated with productive-cicatrificial lesions of the pulmonary artery than with the same lesions of the aorta. The anatomic character of the lesion, as it affects both the aorta and the pulmonary artery, is essentially the same, although it is probable that in the pulmonary artery the internal elastic lamina is more severely affected than in the aorta. Both the aorta and the pulmonary artery show productive-cicatrificial lesions in about half of the cases, but this does not of necessity mean that the disease in one vessel is an extension from the lesion in the other vessel. The disease affects the sexes about equally, and has been found at the age extremes of 28 and 58 years, with the maximum incidence in the sixth decade. It is associated with cardiac difficulties and ultimately congestive heart failure, and may exhibit a fairly characteristic roentgenogram. The most frequent murmur is a systolic murmur over the pulmonic area. Occlusion of a main branch of the pulmonary artery appears to have some relation to development and progress of pulmonary tuberculosis on the affected side.

AUTHOR'S SUMMARY.

**HYPERPLASTIC SCLEROSIS OF THE PULMONARY ARTERY AND ARTERIOLES.** MARTIN J. SOKOLOFF and HAROLD L. STEWART, Arch. Int. Med. **51**:403, 1933.

A case of hyperplastic sclerosis of the pulmonary artery and arterioles is reported. As an etiologic factor syphilis was as well ruled out as possible. The history was not significant. The patient had no scars on the penis, and he had never had antisyphilitic treatment. The Wassermann and Kahn reactions of the

blood were negative. The patient's wife had had five children and no miscarriages. All the children were living and healthy, and none showed any stigma of hereditary syphilis. There were no suggestive clinical manifestations, and roentgen studies of the patient's skeleton revealed no syphilitic lesions. After death no gross or microscopic evidences of the disease were found in the tissues. No spironemas could be demonstrated, although diligently searched for. In the sections of the pulmonary artery and arterioles there were no newly formed capillaries, round cell infiltrations in the adventitia and media or proliferative endarteritis of the vasa vasorum, characteristic of syphilitic arteritis. The associated factors of etiologic importance were a prior attack of influenzal pneumonia, increased epinephrine in the circulating blood, stimulation, probably reflexly, of the nerves supplying the pulmonary artery and asthma. The term "Ayerza's disease" should not be used synonymously with sclerosis of the pulmonary artery, since the pathologic process may be present for years before the complex of symptoms which Ayerza described make their appearance.

AUTHORS' SUMMARY.

PALATAL MYOCLONUS. WALTER FREEMAN, *Arch. Neurol. & Psychiat.* 29:742, 1933.

Freeman describes two cases of rhythmic movements of the pharynx and soft palate which could be interrupted momentarily by phonation and which did not interfere with speech. One patient was alcoholic, with a blood pressure of 244 systolic and 124 diastolic; the other one had marked arteriosclerosis. The pathologic changes were numerous. The most interesting lesions were found in the central tegmental fasciculus and the homolateral olivary body; there was "hypertrophic" degeneration of the homolateral olivary body.

G. B. HASSIN.

ALCOHOLIC ENCEPHALOPATHY. LAURETTA BENDER and PAUL SCHILDER, *Arch. Neurol. & Psychiat.* 29:990, 1933.

The twenty-four cases of chronic alcoholism reported by Bender and Schilder represent a variety of syndromes, such as Korsakoff's polyneuritic psychosis, delirium, cerebellar manifestations, acute catatonic states and other forms. Some of the cases have been studied morphologically. The authors concede that the term polioencephalitis, which means an inflammatory condition, is not justified by the type of the histologic changes, and that "alcoholic encephalopathy" is more suitable. The histologic changes described by them were considered constant—ependymitis and subependymal gliosis with a manifest if not exclusive involvement of the gray matter, especially around the ventricles and at the base, including the mamillary bodies. Other striking changes were vascular—hyperemia of the blood vessels, proliferation of the capillaries and occasionally hemorrhages. According to Bender and Schilder, the cause of the central nerve changes is in the spinal fluid, which is filled with toxins.

G. B. HASSIN.

DIFFUSE SCLEROSIS WITH PRESERVED MYELIN ISLANDS. K. LÖWENBERG and T. S. HILL, *Arch. Neurol. & Psychiat.* 29:1232, 1933.

The authors' patient was a man, aged 54, who had been sick for eleven years with signs of lesion of the pyramidal tract, intention tremor of the upper extremities, unsteady gait and mental deterioration, which was progressive. There was diffuse sclerosis, as seen in Schilder's disease, with numerous perivascular islands of preserved myelin, as described in Pelizaeus-Merzbacher's disease. There was also demyelination of the optic thalamus and globus pallidus, while the medulla and pons were normal. The cerebellum was only slightly involved. As the case clinically was not one of Pelizaeus-Merzbacher's disease, the authors classify it as a subdivision of diffuse sclerosis.

G. B. HASSIN.



MORPHOLOGIC CHANGE IN FAT TISSUE PRODUCED BY INSULIN IN MALNUTRITION. H. BLOTNER, J. A. M. A. **100**:1235, 1933.

After a patient suffering from nondiabetic malnutrition had gained 21 pounds (9.5 Kg.) on insulin treatment, the depth of the subcutaneous fat of the abdomen had increased from 3 to 16 mm., and the average diameter of each fat cell from 35 to 79.8 microns. Before the administration of insulin the fat cells appeared small with a thick wall. After the gain in weight, the fat cells became large and distended so that the walls looked like a thin membrane. There appeared to be an increase in the deposit of fat.

FROM AUTHOR'S SUMMARY.

CONGENITAL HEART BLOCK. W. M. YATER, J. A. LYON and P. E. McNABB, J. A. M. A. **100**:1831, 1933.

A review of the literature reveals records of forty-four acceptable cases of congenital heart block, including the present report. Certain criteria have been applied in collecting these cases. In only five cases have necropsies been performed, and in only three of these have histologic studies of the conduction system been made. A congenital defect of the bundle of His appears to be the cause of this condition in most cases. The most common clinical diagnosis is patent interventricular septum. In the case here reported, there was a defect in the interventricular septum with almost complete congenital absence of the bundle of His. The case is of great significance not only as a completely studied one of congenital heart block, but also as an example of the rarest form of interventricular septal defect.

AUTHORS' SUMMARY.

LESIONS OF THE NERVOUS SYSTEM IN RATS ON A DIET LOW IN VITAMIN A. H. M. ZIMMERMAN, J. Exper. Med. **57**:215, 1933.

Under the conditions of these experiments, which consisted essentially of maintaining rats on a ration adequate in all dietary essentials as far as is known except vitamin A, the following changes were produced in the nervous system: Degeneration of the medullary sheaths of the brachial plexuses and sciatic nerves, and less often of the vagus nerves. Such lesions were not found in the optic nerves. Degeneration of the medullary sheaths of the sensory tracts on the periphery of the spinal cord and in the posterior columns. Much less frequently similar lesions were found in both the crossed and the uncrossed pyramidal tracts. Changes of the same nature were found in the posterior nerve roots and less frequently in the anterior nerve roots of the spinal cord. Evidence was adduced to indicate that the changes in the sensory tracts of the spinal cord followed those in the posterior nerve roots. With the onset of muscular weakness and incoordination in these animals anatomic changes like those just described were found at necropsy, but they were not present for any appreciable period preceding the onset of these clinical signs. For a short but undetermined period following clinical signs of recovery from the nervous disease, marked lesions were still present in the nervous system at necropsy. These lesions in the nervous system were produced by a ration containing no cereals which might have contributed a "toxic" substance to account for the degeneration of the myelin sheaths. Neither does a deficiency in unsaturated fatty acids appear to have played a rôle in their development.

AUTHOR'S SUMMARY.

THE SUBCUTANEOUS NODULES IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS. M. H. DAWSON, J. Exper. Med. **57**:845, 1933.

A comparative study on the subcutaneous nodules in rheumatic fever and rheumatoid arthritis was presented as part of an investigation which has been conducted in this clinic on the relationship that exists between these two clinical entities. It is believed that the present study has shown that these subcutaneous nodules are highly characteristic of the two diseases and that they represent different phases of the same fundamental pathologic process. However,

it should be pointed out that the presence of closely related or even identical lesions in two separate clinical entities cannot be considered as valid evidence in support of the hypothesis that the two diseases are etiologically related. Comparative clinical studies on the relationship of rheumatic fever and rheumatoid arthritis will be presented in a succeeding communication. These studies, as well as serologic investigations on the two diseases which have been reported elsewhere, lend further support to the conception that rheumatic fever and rheumatoid arthritis are intimately related and possibly different responses of affected persons to the same etiologic agent.

AUTHOR'S SUMMARY.

OXYCEPHALY IN MAN AND RABBIT. H. S. N. GREENE, J. Exper. Med. **57**:967, 1933.

Craniosynostosis and the pathologic complex with which it is associated in the rabbit are not identical with the abnormalities seen in man; but apparently the two sets of conditions have enough in common to warrant the conclusion that they probably have a common mode of origin. The evidence so far obtained shows that in the rabbit these conditions are hereditary. The factors for the production of the cranial abnormalities are widespread, but the occurrence of deformities is comparatively rare in a general animal population. Inbreeding and selective matings, however, tend to increase their frequency, and also the frequency of other abnormalities with which they may be associated, either by chance or by close genetic relationship in the germ plasm. In this way, a pathologic complex is built up and perpetuated. So far as the cranial abnormalities are concerned, it seems now that oxycephaly and related conditions are variations resulting from a displacement or division of primary ossification centers controlled by hereditary factors.

AUTHOR'S SUMMARY.

THE LESIONS CAUSED BY CERTAIN NEUROTOXIC PHENOL ESTERS. R. D. LILLIE and M. L. SMITH, Nat. Inst. Health Bull., no. 160, 1932, p. 54.

A study has been made of the lesions of the nervous system of cats produced by a group of phenol esters which give rise to characteristic neurologic symptoms after a latent interval of several days' duration.

The compounds producing such delayed neurotoxic action in cats are triphenyl phosphate and triorthocresyl phosphite in addition to the previously described triorthocresyl phosphate.

It is shown that the lesions produced in cats by triphenyl phosphate are mainly a degeneration of the nerve cells in the acute type of poisoning, and degeneration of the peripheral nerves in the subacute types of poisoning.

The lesions produced by triorthocresyl phosphite are shown to consist of combined system degeneration involving ascending spinocerebellar tracts and descending mesencephalic-pontile-cerebellospinal tracts, in addition to the minor degeneration of the lower motor neuron which is characteristic of the action of triorthocresyl phosphate.

AUTHORS' SUMMARY.

BONE MARROW IN THE LUNGS. G. DOLFINI, Pathologica **25**:38, 1933.

Normal marrow was found in the lumen of the pulmonary artery and its branches in a rabbit which died of tuberculosis.

E. VON HAAM.

CHANGES IN THE PANCREAS IN PREGNANCY. K. ROSENLOECHER, Arch. f. Gynäk. **151**:567, 1932.

In pregnancy the islands of Langerhans are increased in number and size. The epithelial cells of the pancreatic parenchyma also are changed, with an increase in the zymogen granules.

POLYPOID GROWTH OF CERVICAL GLANDS IN THE VAGINA DURING PREGNANCY.  
HANS ZACHERL, Arch. f. Gynäk. **153**:224, 1933.

In a woman ten weeks pregnant, the vagina presented a peculiar polypoid appearance owing to a growth in the wall of the cervical glands, which was thought to have originated in embryonic remains of epithelium from Müller's duct.

JACOB KLEIN.

LESIONS IN THE PULMONARY ARTERY IN RHEUMATISM. HERMANN CHIARI,  
Beitr. z. path. Anat. u. z. allg. Path. **88**:1, 1932.

Autopsy revealed in the pulmonary artery mucoid degeneration and swelling of the fibrous stroma, leading in some cases to necrosis of the muscular tissue but never to degeneration of the elastic lamellae. Besides these nonspecific changes focal cell proliferations and infiltrations of the media with large cells were noticed. Chiari regards these cell infiltrations, which were mostly grouped around the vasa vasorum, as specific rheumatic lesions. In these areas the elastic fibrils were torn or completely degenerated. These histologic changes explain the relative pulmonary insufficiency from which these patients suffer during life.

C. ALEXANDER HELLWIG.

HISTOLOGIC CHANGES IN THE BLOOD VESSELS IN ALLERGIC CONDITIONS AND THEIR RELATIONSHIP TO PERIARTERITIS NODOSA. WALTER METZ, Beitr. z. path. Anat. u. z. allg. Path. **88**:17, 1932.

In white rats, the histologic picture of periarteritis nodosa was produced by repeated injections of hemolytic streptococci. The same lesions followed injection of beef serum. Periarteritis is therefore regarded as not always due to bacteremia and local immune reactions, but possibly caused also by proteins. The experiments indicate that the primary cause of periarteritis nodosa is a peculiar reaction of the endothelial cells. The histologic changes in the blood vessels of some of the animals resembled rheumatic lesions.

C. ALEXANDER HELLWIG.

MUCOUS GRANULOMAS IN THE SALIVARY GLANDS AND IN THE MUCOSA OF THE STOMACH. H. HAMPERL, Beitr. z. path. Anat. u. z. allg. Path. **88**:193, 1932.

Mucus is often found in the stroma of the salivary glands and in the mucosa of the pyloric portion of the stomach. These deposits of mucus outside of the lumens of the glands are seldom due to inflammatory destruction of the glands. The most common cause is a traumatic rupture of dilated mucous glands. Around these masses, a peculiar granulation tissue forms which encapsulates and finally resorbs the mucus.

C. ALEXANDER HELLWIG.

MORPHOLOGIC CHANGES IN THE HEMATOPOIETIC ORGANS FOLLOWING ACUTE LOSS OF BLOOD. OSCAR LEWIN, Beitr. z. path. Anat. u. z. allg. Path. **88**:349, 1932.

Rabbits were killed after bleeding at periods varying from five minutes to twenty-four days. Two fifths of the total blood volume was removed. In contradistinction to pernicious anemia, acute loss of blood is not followed by erythropoiesis in other organs than the bone marrow. Only in the spleens of experimental animals was a moderate degree of blood formation noticed. There were no morphologic changes in the suprarenals, kidneys, lungs and thymus gland. In the liver, hypertrophy of the Kupffer cells was observed, and in the lymph glands, a marked proliferation of the reticulo-endothelial cells.

C. ALEXANDER HELLWIG.

EARLY POSTMORTEM CHANGES OF THE MEGAKARYOCYTES IN THE MARROW OF THE RABBIT. W. N. MARGOLIN, Beitr. z. path. Anat. u. z. allg. Path. **88**:362, 1932.

The cytology of the bone marrow of twenty-nine rabbits was studied before and after death. As early as one-half hour after death, pyknosis of the nuclei of the

megakaryocytes could be demonstrated. The number of pyknotic cells increased with the interval between death of the animal and examination. After from four to five hours, all megakaryocytes were pyknotic. At this time all the other cells of the bone marrow showed a normal structure. These findings indicate a marked lability of the megakaryocytes.

C. ALEXANDER HELLWIG.

LIPOID SUBSTANCES OF THE BLOOD PLASMA IN BLOOD VESSELS OF DIFFERENT ORGANS. MAXIMILIAN MANDELSTAMM, *Beitr. z. path. Anat. u. z. allg. Path.* 88:377, 1932.

Sudanophil substances are frequently found in the blood plasma, especially in the brain. Of seventy-four brains examined, fifty-five showed positive findings. The lipid substances are also frequently present in the ovaries, thyroid gland, hypophysis and pancreas, while they are less common in the kidneys and are exceptional in other organs. The presence of sudanophil substances in certain organs cannot be explained on the basis of general abnormal conditions of the whole blood, but is most likely due to particularities in the circulation of the blood. The lipid substances are most frequently found in the blood vessels that contain exclusively, or almost exclusively, blood plasma. They were most abundant in the organs of patients with acute infections, causing disturbance of the peripheral circulation.

C. ALEXANDER HELLWIG.

ASEPTIC INFLAMMATION AFTER BLOCKING LOCALLY THE RETICULO-ENDOTHELIAL SYSTEM. A. N. TSCHISTOWITSCH, *Beitr. z. path. Anat. u. z. allg. Path.* 88:426, 1932.

Trypan blue was injected into rabbits subcutaneously, three times within four days. On the fifth day, at the place of injection, a small piece of sponge was implanted under the skin. The foreign body, together with the surrounding tissue, was removed at different intervals and examined histologically. The histiocytes were laden with coarse particles of the intravital stain, and they were found in the region of the foreign body, but had not invaded the sponge. The histiocytes had apparently emigrated from the surrounding connective tissue and from blood vessels. There were no transitions recognizable between fibroblasts and histiocytes. The endothelial cells were without stain. If in such experiments india ink is used instead of trypan blue the stain is not so readily engulfed by the histiocytes. Large amounts of india ink were found free in the interstitial stroma.

C. ALEXANDER HELLWIG.

HISTOLOGY AND ETIOLOGY OF SUBACUTE YELLOW ATROPHY OF THE LIVER IN CHILDREN. R. BENEKE, *Beitr. z. path. Anat. u. z. allg. Path.* 88:538, 1932.

The author gives a detailed microscopic description of the internal organs of a child who died of severe jaundice. In the liver, there was almost complete necrosis of the parenchyma. Only a few fatty, degenerated liver cells were preserved. There was proliferation of the bile ducts and of the interlobular stroma. Chemical examination of the internal organs did not reveal any poison, and the history was not in favor of poisoning as the etiologic factor. The author hypothecates that the necrosis of the liver was possibly caused by an intensive spasm of the hepatic artery.

C. ALEXANDER HELLWIG.

LESIONS OF THE PULMONARY ARTERY DUE TO SYPHILITIC MESAORTITIS. JULIUS F. BUCHALY, *Beitr. z. path. Anat. u. z. allg. Path.* 89:1, 1932.

In eighteen of the twenty-eight cases of syphilitic mesaortitis, the pulmonary artery was compressed by aneurysm of the ascending branch of the aorta. Adhesions between the aneurysm and the pulmonary artery may produce pressure atrophy of the wall. In ten cases, the syphilitic inflammation had spread from the aorta

to the pulmonary artery, involving also the media. Five cases showed a perforation of the aorta into the pulmonary artery. The syphilitic inflammation may follow the ductus Botalli.

C. ALEXANDER HELLWIG.

DISEASES OF THE LUNGS IN THE NEW-BORN, ASSOCIATED WITH THE ASPIRATION OF AMNIOTIC FLUID. FRANZ SZLÁVIK, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:40, 1932.

Aspirated amniotic fluid is commonly found in the lungs of the new-born. The anatomic findings following aspiration of the fluid depend on the chemical constituents and the amount of aspirated fluid, the duration of birth and the length of extra-uterine life. They consist in a more or less severe inflammation of the pulmonary tissue, which by itself, however, is never fatal, but heals as a rule within the first week. Aspiration of infected fluid was observed in a stillborn child, the mother having died of streptococcic metritis. Infection of the lungs in the new-born can be due to aspiration of vaginal secretion during birth, to aerogenous infection or to the aspiration of vomitus. It may lead to fatal lobular pneumonia. In one of the author's cases the pneumococcus type IV was the infecting organism. Fetal atelectasis is recognized by the presence of myelin in the alveoli. It forms from degenerated alveolar epithelium.

C. ALEXANDER HELLWIG.

AN UNUSUAL CASE OF CHRONIC FOREIGN BODY PNEUMONIA CAUSED BY GRAIN DUST. HILDE BÖRGER, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:135, 1932.

A woman, aged 57, died after she had suffered for nine years from bronchitis and chronic pneumonia. She had contracted the disease while watching, for from two to three hours, the operation of a thrashing machine. At autopsy, both lungs showed marked carnification and scar formation, bronchiectasias and pleural adhesions. Many giant cells were found in the pulmonary tissue surrounding foreign bodies, giving the chemical reactions typical of cellulose.

C. ALEXANDER HELLWIG.

OSTEOSCLEROTIC PSEUDOLEUKEMIA. CHARLES WOLF, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:151, 1932.

A case of osteosclerosis associated with myelogenous pseudoleukemia was studied macroscopically and microscopically at autopsy. The ribs, sternum, vertebrae and femur showed marked sclerosis of the medullary cavity. The sclerosis is only rarely the final stage of leukemia or pseudoleukemia, but is due to premature atrophy of the bone marrow and compensatory extra-osseous hematopoiesis in the spleen and liver. Since in osteosclerosis the formation of blood cells is active only in the liver and spleen, radiation treatment or splenectomy is contraindicated in pseudoleukemia when roentgen examination of the skeletal system reveals an association with osteosclerosis.

C. ALEXANDER HELLWIG.

THE GERMINAL CENTERS OF FLEMING. A. V. ALBERTINI, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:183, 1932.

The effect of the x-rays, arsenic and lead on the lymph nodes was studied in guinea-pigs and white mice. Following irradiation, diffuse degeneration was noted in the lymphoid tissue of the primary nodules and in the germinal centers, while after arsenic and lead poisoning, only the cells of the germinal centers were destroyed. The lesions in the germinal centers following poisoning are explained by the particular arrangement of the blood vessels in the lymph nodes. The regeneration of lymphocytes after irradiation is not limited to the germinal centers, but is more active in the primary nodules. Therefore, the theory of Flemming, who regards the germinal centers as hematopoietic organs, is not accepted. In the author's opinion, the germinal centers serve as filters in which various poisons brought with the blood stream from other regions of the body are taken up.

C. ALEXANDER HELLWIG.

SPONTANEOUS CEREBRAL HEMORRHAGE. K. WOLFF, Beitr. z. path. Anat. u. z. allg. Path. **89**:249 and 487, 1932.

The histologic changes in brain tissue and blood vessels after traumatic injuries of the brain were studied to form a basis of comparison with the findings by Westphal, Bär and Schwartz, who advocate the modern functional theory of apoplexy. In traumatic destruction of the brain, necrosis of the wall of the blood vessels was observed only in the regions of hemorrhage. Since these angionecroses develop several hours after the injury, they are apparently not the cause of spontaneous hemorrhage, as Schwartz and others hypothesize. Proteolytic enzymes of leukocytes play an important rôle in these vascular changes; probably, also, ferments liberated from destroyed glia cells act on the wall of the vessel. Fat-laden phagocytes appeared not before thirty-six hours after the injury. On the second day, proliferating spindle cells were found in capillaries, and the first collagenous fibers were demonstrated nine days after the accident.

In three of seven brains in cases of fatal apoplexy, ruptured blood vessels were found in the center of the cerebral hemorrhage. The walls of the ruptured blood vessels showed hyaline sclerosis and defects in the elastic layer. In one case in which all the ventricles were filled with blood a ruptured aneurysm was found. In the brains of elderly people, the arteries of the corpus striatum frequently show severe degeneration of the walls, and a rupture may easily occur when the blood pressure is suddenly increased. The author concludes that the classic doctrine of rupture of the blood vessels as a cause of spontaneous cerebral hemorrhage still holds, and that it is better based on facts than the modern theory of functional circulatory disturbances causing apoplexy.

C. ALEXANDER HELLWIG.

SIGNIFICANCE OF LESIONS OF THE HEART MUSCLE IN ANGINA PECTORIS.

FRANZ BÜCHNER, Beitr. z. path. Anat. u. z. allg. Path. **89**:644, 1932.

Large longitudinal sections of the hearts in ten cases of angina pectoris were made, the technic of Christeller being used. In all of the cases in which death occurred shortly after a paroxysm, smaller or larger focal necroses were demonstrated in the muscle of the heart. These necroses were preeminently located in the inner layer of the wall of the left ventricle, involving also the papillary muscle and trabeculae. The size of these lesions increased with repeated attacks. Büchner concludes that ischemia and necrosis of the heart are responsible for the attack of pain. Coronary sclerosis and syphilis can produce angina pectoris without spasm of the blood vessels by interfering with dilatation of the coronary arteries during increased action of the heart.

C. ALEXANDER HELLWIG.

THE CORONARY CIRCULATION AND ANGINA PECTORIS IN RELATION TO CORONARY OCCLUSION. W. KOCH and L. C. KONG, Beitr. z. path. Anat. u. z. allg. Path. **90**:21, 1932.

The histopathology of coronary occlusion was studied by means of serial sections of the involved vessels. The results of such a study of seven selected cases are presented. The Spalteholz method of cleared injection preparations was found useful in the investigation of the coronary circulation in disease of the coronary arteries. Twenty-nine hearts of persons who had had anginal attacks form the basis of an attempt to correlate coronary arterial disease with angina pectoris. Coronary stenosis and occlusion may be due to atheroma, fibrous thickening or thrombosis. The three processes are often combined, but the predominance of one or the other process may yield information relative to the duration and character of myocardial changes. Soft atheroma is the most common cause of occlusion, and may occur suddenly as the result of acute degeneration and swelling of an atheromatous nodule. Calcifying senile atherosclerosis has relatively little harmful effect on the muscle of the heart. Although the occurrence of syphilitic disease of the coronary arterial system is admitted, syphilis is of importance chiefly because

it leads to stenosis of the mouth of the coronary artery as a part of syphilitic involvement of the root of the aorta. The effects of occlusion depend on the collateral circulation that is developed in association with coronary disease. Anastomoses are richest between the right coronary artery and the circumflex branch of the left. The descending branch of the left coronary artery has fewest anastomoses, except at its terminal portion, where it unites with the right coronary artery on the posterior surface of the apex of the heart. Infarction occurs most often, therefore, in the territory of distribution of the left descending artery, but is of least functional significance here. Occlusion occurs most often in the proximal portion of the left descending artery and in the distal portions of the circumflex and right descending arteries. In cases with a history of angina pectoris, infarction was a prominent feature, and usually two main vessels were stenosed or occluded. Stenosis of one vessel with an inadequate collateral circulation through another main branch usually leads to acute cardiac insufficiency rather than to anginal attacks. Syphilitic stenosis of the mouth of a coronary artery is a gradual process, which usually permits the development of an adequate collateral circulation; hence infarction is rare. Since the circulation in such cases is through a single coronary artery, sudden death or anginal attacks may occur as the result of myocardial ischemia if excessive demands are made on the coronary circulation.

O. T. SCHULTZ.

HISTOLOGY OF THE GASTRIC MUCOSA AND ACUTE EROSIONS. · H. HAMPERL, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:85, 1932.

This is a systematic study of the human gastric mucosa undertaken for the purpose of evaluating changes that are frequently seen and of determining whether such changes are inflammatory. Stomachs obtained within six hours after death were examined. A strip of mucosa 1 cm. wide, taken along the lesser curvature of the fixed stomach from the cardia to the pylorus, was rolled into a spiral, embedded and sectioned. Two other strips were taken at right angles to this, one across the fundus, the other across the antrum. Great reliance was placed on the oxidase stain for detecting the presence of leukocytes. A modification of Graeff's oxidase method is given for the selective staining of eosinophilic leukocytes. Eosinophilic and neutrophilic leukocytes were found so constantly in the mucosa as to be physiologic or normal. These cells migrate through the mucosa and were seen in the mucus covering the surface of the epithelium. Only when the presence of such cells is associated with changes in the surface and gland epithelium is it proper to speak of inflammation. Inflammation, or true acute gastritis, usually leads to the formation of minute erosions. In these erosions the surface epithelium is injured and then attacked by the gastric juice. Beneath this superficial zone of necrosis is a second zone of fibrinoid necrosis, and beneath this a zone of leukocytic infiltration in which leukocytes are more numerous than in the normal gastric mucosa. The accumulation of leukocytes is held to be secondary to the necrosis of the more superficial tissue.

O. T. SCHULTZ.

### Microbiology and Parasitology

DETERMINATION OF TUBERCLE BACILLEMIA BY LÖWENSTEIN'S METHOD. JOSEPH SCHRAMKE, *Beitr. z. Klin. d. Tuberk.* **82**:606, 1933.

Apparently tubercle bacillema is an infrequent occurrence in tuberculosis, for in only 2 of 322 cases of pulmonary tuberculosis in which 522 blood cultures were performed was it possible to demonstrate tubercle bacilli. In none with extra-pulmonary complications were they found. Nevertheless, experiments proved that Löwenstein's method is trustworthy in revealing the presence of tubercle bacilli in blood.

AARON EDWIN MARGULIS.

EXPERIMENTAL REINFECTION OF GUINEA-PIGS WITH THE VIRUS OF SPOTTED FEVER. KENSABURO TAKAHASHI and SOYO HOSHIZAKI, *Zentralbl. f. Bakt.* (Abt. 1) **121:39**, 1931.

Forty-six guinea-pigs were reinfected with the virus of spotted fever at intervals of from one to one hundred and twenty-seven days after they had become free from the fever produced by the first infection. Ten showed symptoms (fever and loss of weight), but the period of incubation was prolonged as compared with the first infection, while the febrile period was irregular and shortened.

PAUL R. CANNON.

EXPERIMENTAL STUDIES OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM. T. TANI, K. SAITO and H. FUNADA, *Zentralbl. f. Bakt.* (Abt. 1) **123:219**, 1932.

Rabbits (thirty) were infected intratesticularly, subscrotally or suboccipitally with syphilitic material (passage strain "VIII") and were treated with neoarsphenamine intravenously from thirty-five to five hundred and thirty-nine days after infection. Keratitis developed in five animals, whereas the others remained symptomless. A negative Wassermann reaction in the serums of eight animals became positive but that of the spinal fluids remained negative. Spirochetes were regularly demonstrable in the blood until after the third injection of neoarsphenamine, but could not be found in the spinal fluid at any time. Spirochetes were demonstrated in the blood, spleen, testes and lymph nodes of all but one of seven rabbits thus examined which had been infected for an average period of six hundred and sixty-five days and had had from eight to nine injections of neoarsphenamine. Spirochetes were found in the brain in only one of the six rabbits whose brains were examined.

Fourteen rabbits infected intratesticularly or suboccipitally were treated with neoarsphenamine and were then inoculated suboccipitally on several occasions with homologous or heterologous strains of syphilitic virus. Four normal untreated rabbits were inoculated similarly. Keratitis or orchitis developed in the latter, whereas the previously infected and treated animals showed no symptoms. The negative Wassermann reaction of the blood serum became positive in three of the four normal rabbits and in three of the fourteen previously infected and treated ones. Spirochetes were demonstrable in the blood of the control animals after the second injection, but could not be found in the treated animals until after the fourth injection. They were not demonstrable in the spinal fluid in either group. Neither could spirochetes be found in the brains of the rabbits from either group.

The authors conclude that the rabbits previously infected and treated with neoarsphenamine showed a definite acquired immunity, although this could be overcome by repeated superinfections. The authors believe that the immunity of the central nervous system to syphilis is unusually high in the rabbit, but they cannot explain this resistance.

PAUL R. CANNON.

SOURCE OF THE GUARNIERI BODIES. E. PASCHEN, *Zentralbl. f. Bakt.* (Abt. 1) **124:89**, 1932.

The author gives opinions as to the source and nature of the Guarnieri bodies in the skin and eye. He believes that many of the cell inclusions are simply pyknotic nuclear fragments, nucleoli and chromatin masses from degenerating leukocytes, but that the bodies which actually contain the virus are very small elements immediately around the nucleus in cells at the site of inoculation.

PAUL R. CANNON.

MORPHOLOGIC DEMONSTRATION OF THE SMALLPOX VIRUS IN TISSUE CULTURE. E. G. NAUCK and E. PASCHEN, *Zentralbl. f. Bakt.* (Abt. 1) **124:91**, 1932.

The authors injected vaccine virus into rabbits' testicles and subjected the latter to tissue culture. They observed a direct correlation between the numbers of Paschen bodies and the virulence of the cultures. These bodies persisted through thirty-five passages in tissue culture, and in some instances yielded positive results



in the skin of rabbits in a dilution of 1:100,000 of the original culture. The authors conclude that these so-called elementary bodies described by Paschen are the carriers of the virus.

PAUL R. CANNON.

EFFECTS OF INHALATION OF EUCALYPTOL ON TUBERCULOUS GUINEA-PIGS INFECTED INTRATRACHEALLY. T. H. AMAKO, Zentralbl. f. Bakt. (Abt. 1) **124**: 95, 1932.

Guinea-pigs were infected intratracheally with tubercle bacilli and were subjected in enclosed cages to treatment by inhalation of eucalyptol until the death of the animals. This treatment had no appreciable effect on the length of life of the infected animals as compared with control animals similarly treated.

PAUL R. CANNON.

CULTIVATION OF THE VIRUS OF SPOTTED FEVER. ILCHUN YU, Zentralbl. f. Bakt. (Abt. 1) **124**:181, 1932.

Passage virus derived originally from the blood of a patient with spotted fever was injected intraperitoneally into a guinea-pig, and the testes and tunica vaginalis were removed aseptically from twenty-four to forty-eight hours later, cut into small pieces and grown in tissue culture. The virus persisted for seventy days, through six generations, in vitro and was demonstrable both in the tissue and in the culture fluid itself. Rickettsial bodies were never found in the tissue culture except when embryonic extract, from embryos of guinea-pigs convalescing from a previous infection, was used.

PAUL R. CANNON.

## Immunology

GROUP-SPECIFIC QUALITIES IN SALIVA AND OTHER BODY FLUIDS AND DEMONSTRATION OF TWO TYPES OF ELIMINATORS OF THE GROUP-SPECIFIC QUALITIES. HAKARU SASAKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:101, 1932.

All persons can be divided into two groups: (a) those who eliminate the group qualities A, B or O in the saliva, gastric juice, milk or urine and (b) those who do not eliminate them. The ability to eliminate is constitutional and does not depend on the blood group. In persons of group AB the two factors always behave identically. The presence of the group qualities in the secreta was established by means of the inhibition of agglutination and hemolysis. The inhibition of the specific anti-O agglutination (in absorbed ox serums) is brought about by secretions of all four groups. The qualities M and N were not demonstrated in the saliva. There was no demonstrable relation between the presence in the saliva of the ferment which is able to destroy blood group qualities and the presence or absence of elimination of the group qualities.

I. DAVIDSOHN.

INHERITANCE OF SEROLOGIC TYPES OF ELIMINATORS OF GROUP-SPECIFIC SUBSTANCES. F. SCHIFF and H. SASAKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:129, 1932.

The authors made a study of 68 families with 215 children and 144 twins. The eliminators of the qualities A, B and O in saliva were designated as S; those failing to eliminate as s. The S and s qualities are inherited as a mendelian pair and are fully developed in infants. The s quality was encountered more frequently in the O group than in the others.

I. DAVIDSOHN.

PURIFICATION OF GROUP SUBSTANCE A OF HUMAN RED BLOOD CELLS. F. OTTENSOOSER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:140, 1932.

The group-specific substances are contained in the stroma and not in the hemoglobin. The inhibiting effect on an antishoop hemolysin produced by injection of

human red blood cells of group A, according to the procedure of Brahn and Schiff, was found the most sensitive reaction for the study of the substance A. The stroma containing the substance A contains no less of the group-specific substance than the red blood cells from which it was prepared. A method of preparation of stroma of high purity with only from 0.5 to 2.5 per cent of hemoglobin is described. Extracts prepared from stroma with a highly diluted sodium hydroxide solution contained a more active A substance than the original stroma, which indicates that the alkali had an activating effect.

I. DAVIDSOHN.

PRODUCTION, PROPERTIES AND ACTION OF CONCENTRATED TOXOID. C. SIEBENMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:167, 1932.

Acetic acid and barium sulphate were used for the precipitation. Only one of three horses gave a satisfactory antitoxin. All three horses showed various degrees of renal damage, and one of them eliminated diphtheria antitoxin in the urine. Rises in temperature were frequent. Siebenmann recommends the exclusion of horses with fever at the beginning of the treatment. There is no reason to recommend replacement of strong unconcentrated toxoid by the concentrated toxoid, but weak toxin can be made useful by concentration.

I. DAVIDSOHN.

INDUCTION OF CHANGES IN THE SPECIFICITY OF IMMUNE SERUMS BY CHEMICAL TREATMENT. F. BREINL and F. HAUROWITZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:176, 1932.

Horse immune serums against paratyphoid bacilli A and B and against dysentery Y and Flexner bacilli were treated according to Landsteiner's method with atoxyl, aniline, metanilic acid, iodine, formaldehyde and sodium hydroxide. The serums were tested before and after treatment for the specific and group agglutinins, and also with an antihorse precipitating serum for species specificity. The antibodies were influenced in different degrees. Iodine destroyed agglutinins but was less harmful to precipitinogens; formaldehyde made the serums highly specific and left the precipitinogens more or less intact. The other procedures had a uniformly destructive effect on the antibodies as well as on the species specificity. Diphtheria antitoxin and typhus convalescent serum were uniformly damaged by all procedures. The conception that antibodies are proteins or that they are closely associated with proteins is supported by a chemical analysis of the experimental results. The aromatic groups of the protein molecule are no less important for the specificity of the antibody than they are for the antigen.

I. DAVIDSOHN.

INACTIVATION OF COMPLEMENT WITH THE POISON OF THE SNAKE BOTHROPS. OTTO BIER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:187, 1932.

The poisons of the snakes Bothrops jararaca (Wied) and Bothrops atrox (L) destroyed the thermostable fourth component of complement. The time of inactivation had to be strictly adhered to. The action on less diluted and on heated serum is different from the action of diluted ammonia, which is known to destroy the fourth component of complement.

I. DAVIDSOHN.

THE FLOCCULATION TIME DURING THE IMMUNIZATION OF HORSES FOR DIPHTHERIA ANTITOXIN. A. J. VAN DEN HOVEN VAN GENDEREN and C. A. KRAMERS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:280, 1932.

Five horses were treated with diphtheria anatoxin. The flocculation time became stationary for many months after an initial rise. After a longer interval between injections, their renewal was in some cases followed by a shortening of the flocculation time; however, there was no quantitative relation between the new flocculation time and the rise of the antitoxin titer. Both the in vivo and the in

vitro titers were determined; the former not infrequently had a tendency to drop below the latter in the course of the treatment. The in vivo titer had a tendency to sink following a large venesection.

I. DAVIDSOHN.

#### NATURAL HEMAGGLUTININS IN SNAKES AND OTHER COLD-BLOODED ANIMALS.

A. DO AMARAL and D. V. KLOBUSITZKY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:315, 1932.

Eight species of snakes, two species of turtles and one species of lizards, as well as the horse, the sheep and the goat, were studied. No auto-agglutinins or iso-agglutinins were found in the plasma of the cold-blooded animals. The plasma of some of the snakes and of one turtle agglutinated occasionally the erythrocytes of one of the lizards. The plasma and erythrocytes of the snakes and the horses did not act on each other. The erythrocytes of one of the species of turtles (*Bufo marinus*) were agglutinated by the plasma of the horses, and, in turn, their plasma agglutinated the erythrocytes of some horses.

I. DAVIDSOHN.

#### THE SPECIFICITY OF FIBRIN. HANS J. FUCHS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:343, 1932.

The precipitate obtained from serum with 2.5 per cent trichloroacetic acid, followed by washing, possessed the same qualities as the purified fibrin which Fuchs employed previously as the reagent in his test for cancer. The test is based on the observations of Freund and Kaminer that normal serum dissolves carcinomatous cells and on those of Weinberg that the normal serum shows an increase of nonprotein nitrogen after such treatment. Fuchs demonstrated the presence of the tumor-specific substance in the fibrin and in the precipitate of the serum. On the basis of 3,400 cases he found it to be tumor specific but not to permit the differentiation of various types of tumors and of their origin. The serum of patients with cancer had no such effect on the tumor substance. Occasionally a decrease instead of an increase of the nonprotein nitrogen was noted in the mixture of serum of patients with cancer and fibrin or precipitate of such patients. This is, according to Fuchs, a sign of developing immunity. The fibrin and the precipitate contain immune bodies of the serum as well as antigenic substances. Antisheep hemolysin was similarly demonstrated in the serum precipitate of immunized rabbits.

I. DAVIDSOHN.

#### ORIGIN OF DIPHTHERIA TOXIN. RICHARD PRIGGE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:421, 1932.

Dried, washed and pulverized diphtheria bacilli have the same toxic effect on guinea-pigs as toxin obtained from cultures. Their toxicity is destroyed by heating at 100 C. and by neutralization with antitoxin. While it was impossible to liberate toxin from the bacilli by means of high pressure, large quantities of the toxin were liberated by suspension in physiologic solution of sodium chloride. The yield of toxin decreased rapidly with repeated washings, but if the bacterial bodies were left alone for a few days considerable quantities of toxin could again be obtained by washing with physiologic solution of sodium chloride. After repeated washings at proper intervals, the bacterial bodies were found to contain only traces of toxin. The ease with which the toxin passes from the bacterial body into the surrounding medium suggested to Prigge that it is a part of the bacterial body and that after the death of the cell it is liberated gradually with progressive disintegration. Prigge checked and disproved the hypothesis of Dernby and Walbum that the diphtheria toxin is not produced in the bacteria but is a result of the action of enzymes, liberated from dead bacterial bodies, on albumoses and peptones of the medium.

I. DAVIDSOHN.

STIMULATING EFFECT OF TAPIOCA ON THE PRODUCTION OF DIPHTHERIA ANTITOXIN. E. W. SKROTZKY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77:443**, 1932.

The work was undertaken to check the contradictory reports concerning the effect of tapioca on immunologic response, first published by Ramon. The ability of tapioca to adsorb diphtheria toxin could not be observed in the animal experiment. Precipitation between toxin which was mixed with tapioca and antitoxin was distinctly more marked than that which occurred when the untreated toxin was used. The increase was assumed to be nonspecific. The local irritating effect of tapioca on the skin was rather mild; necrosis was not observed. The formation of a depot in the inoculated area and the increase of the antigenicity recommend the employment of tapioca as an admixture to diphtheria toxin.

I. DAVIDSOHN.

GROUP-SPECIFIC DIFFERENTIATION OF PLACENTAL ORGANS. HORST REICH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77:449**, 1932.

The decidua parietalis (vera) of mothers belonging to group A always contains the quality A, while the decidua basalis contains little of it. The amnion of children belonging to group A contains the quality A, but in varying amounts. The chorion frondosum in the cases of children of group A lacks the quality A. The quality A can be removed from the tissues by proper procedures. In a few cases in which the mother belonged to group O or B and the child to group A the quality A was found in the decidua parietalis. Reich explains that finding by imbibition from the amnion. The placenta proper is considered a zone of group neutrality. The discovery by Schiff and Weiler of a ferment in the placenta which is able to destroy the blood group-specific qualities may be the explanation.

I. DAVIDSOHN.

ACTION OF FECAL EXTRACTS ON ANTIGENS. M. EISLER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77:472**, 1932.

Extracts of human feces destroyed blood group-specific substances in human red blood cells and secretions and in Witte's peptone. Such extracts did not affect precipitinogens of human and horse serum and of paratyphoid bacilli and the Forssman antigen of horse kidney. Fecal extracts of the sheep, dog and rat destroyed the substance A of Witte peptone, but the destructive effect of the feces of rabbits and goats was slight or absent.

I. DAVIDSOHN.

SOME QUALITIES OF THE THERMOSTABLE COMPONENTS OF COMPLEMENT. T. MISAWA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77:477**, 1932.

The report of Gordon, Whitehead and Wormald concerning the presence of the third component in the globulin, and of the fourth in the albumin, fraction was confirmed in principle, although such clearcut separation was seen only occasionally. In most cases each component was predominantly associated with the corresponding fraction. The separation of the thermolabile midportion and end-portion is entirely independent of the separation of the thermostable fractions. The destruction of the third component by cobra poison does not take place if the complement was previously heated at 55 C., or if it was treated with hydrochloric acid in a hypertonic solution. The destruction of the fourth component by ammonia is not affected by these procedures.

I. DAVIDSOHN.

ANAPHYLAXIS AND IMMUNITY. S. METALNIKOV, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77:488**, 1932.

The defense reactions of immunity and the shock of anaphylactic hypersensitiveness are essentially identical phenomena of the hypersensitive organism.

The hypersensitiveness is brought about by the first introduction of the antigen, and the different manifestations of the two conditions are explained by the small quantity of the reintroduced antigen in the case of immunity reactions and by the excessive dose in the case of anaphylaxis. Caterpillars of *Galleria melonella* were inoculated with 0.1 cc. of a culture of *Vibrio cholerae*. After from twenty-four to forty-eight hours a reinjection of a minimum lethal dose had no bad effects, though control insects were killed within from fifteen to twenty-four hours. Caterpillars similarly immunized were reinoculated with large doses of *Vibrio cholerae*. They became very ill within from ten to fifteen minutes and died from two to three hours later. Normal caterpillars given similar large doses appeared normal in from two to three hours and died from eight to ten hours after the injection. An experiment yielding identical results was made with guinea-pigs treated with *Vibrio cholerae*.

I. DAVIDSOHN.

THERMOSTABILITY AND THE NATURE OF ANTIBODIES. L. A. SILBER and M. W. DEMIDOWA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:504, 1932.

Increase in the thermostability of antibodies is due to prevention of coagulation and of denaturing of proteins. Prevention of coagulation preserves only the H agglutinins; prevention of the denaturing action of heat preserves the H and the O agglutinins. Dilution of the serum has an anticoagulating effect, while saccharose acts as an antidenaturant. Heat exerts at the same time a denaturing effect on proteins and a destructive effect on antibodies. The two effects are similar and parallel. This suggests that antibodies are protein substances or are closely associated with them.

I. DAVIDSOHN.

FURTHER STUDIES ON THE THERMOSTABILITY AND NATURE OF THE COMPLEMENT. L. A. SILBER and A. S. SCHAFRAN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:514, 1932.

Antidenaturants, such as saccharose, dextrose, maltose and sorbite, in certain definite concentrations, increased the thermoresistance of complement, enabling it to stand heating at 56 C. for fifteen minutes. Anticoagulants without, or with slight, antidenaturing properties failed to protect. A close relationship was observed between the destruction of the complementary properties of the serum and the denaturing of its proteins.

I. DAVIDSOHN.

SYNTHETIC LECITHIN AND ITS ANTISERUMS. HERTA MAIER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:1, 1933.

The serums of rabbits treated with a mixture of distearyl lecithin and hog serum possessed specific antibody functions with the homologous antigen and with a commercial lecithin. A lecithin antiserum also reacted with distearyl lecithin. The distearyl lecithin antiserum precipitated with the commercial lecithin but not with the homologous antigen. The serum reacted strongly and selectively with lecithin obtained from the brain of the ox, sometimes even more strongly than with the homologous antigen, thus making it advisable to employ the brain preparation, particularly after the addition of cholesterol, as a reagent for the testing of distearyl lecithin antisera. This reactivity was not organ specific, as shown by tests with alcoholic extracts of brain tissue. Sometimes, though not regularly, complement was fixed with the acetone-insoluble fraction of the alcoholic brain extract.

I. DAVIDSOHN.

INFLUENCE OF FORMALDEHYDE ON DIPHTHERIA TOXIN. I. S. SCHMIDT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:27 and 323, 1933.

The rapidity with which diphtheria toxin changes into anatoxin increases with the concentration of formaldehyde, and with a rise in temperature and in the

hydrogen ion concentration, and the change may become almost immediate with high concentrations of formaldehyde. Together with the change in toxicity, which is not reversible, the resistance to heat, acids, alkalis and other chemical influences increases. Because of the marked thermal instability of purified toxin, Schmidt suggests first keeping the mixture of purified toxin and formaldehyde at 0 C. and then increasing the temperature, or changing the raw toxin into anatoxin and then purifying the finished product. The immunizing properties of anatoxin depend not only on its flocculating ability but on other, as yet undetermined, factors which may be due to the presence of extraneous inhibiting or stimulating substances in the medium. The technic employed in the treatment of toxin with formaldehyde may also be responsible.

I. DAVIDSOHN.

ACTION OF FORMALDEHYDE ON DIFFERENT ANTIBODY FUNCTIONS. H. BRAUN. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 78:46, 1933.

The various antibody functions of different types of immune serums are influenced differently by formaldehyde. In a Forssman heterophilic antiserum the complement-fixing antibodies were destroyed by certain concentrations of formaldehyde which did not affect the precipitins. The effect on an antsheep serum was similar; in this case the agglutinins proved more resistant than the hemolysins. The effect of formaldehyde was directly proportionate to the duration of its action, to its concentration and to the temperature. In syphilitic serums the complement-fixing and the precipitating qualities were affected in a uniform manner. In protein antisera (produced by injection of serum) the complement-fixing qualities were more resistant than the precipitins. The species-specific heteroagglutinins anti-O in rabbit immune serums were less resistant than the group-specific agglutinins directed against the quality A.

I. DAVIDSOHN.

THE FRACTIONS OF COMPLEMENT: I. THE FOURTH AND FIFTH FRACTIONS. T. TODA and B. MITSUSE. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 78:62, 1933.

Toda and Mitsuse confirm the existence of the fourth fraction of complement. This fraction is destroyed by chloroform and ether as well as by ammonia and its salts. The technic of preparation of different fractions is described. A fifth fraction was demonstrated, which is destroyed by benzine, and which possesses the highest degree of thermostability of all the fractions, as it stands boiling for one hour. It is recovered in the albumin fraction of the serum, as is the fourth fraction, while the third fraction is attached to the globulin. A comparison of the thermal resistance of the different fractions showed that it rose from the mid-fraction through the end-fraction—from the third to the fourth and to the most resistant fifth fraction. The poison of a viper destroyed the fourth fraction. The same effect was produced by cadmium chloride, known to react readily with lecithin. The fractions destroyed by ether and benzine could be recovered by evaporating the extract and adding the residue to the inactivated serum. The fourth and fifth fractions are assumed to be lipoidal or closely attached to lipoids.

I. DAVIDSOHN.

INFLUENCE OF PHYSICO-CHEMICAL FACTORS ON HETEROPHILIC ANTIBODIES. ILSE SACHS. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 78:122, 1933.

Rapid dilution of the antigenic extract renders it more sensitive if small amounts of the extract are employed, and particularly if the time of the primary incubation is shortened. When larger quantities of the extract are used, the fractional dilution may, under proper conditions, offer advantages. The heterophilic precipitation reaction was considerably increased by incubator temperature. Shaking of the test tubes hastened and intensified the precipitation, but did not affect the

complement fixation. The complement-fixing and hemolytic functions of heterophilic and immune serums, which under the usual conditions are lost by heating to 65 C., could be demonstrated by the use of a very slow fractional dilution of the extract, by prolonged (eighteen hour) icebox fixation of the mixture (antigen, heated antiserum and complement) and by lowering of the sodium chloride concentration (0.6 per cent) during the primary incubation. The precipitating antibodies were absorbed from the fresh and from the heated serum by sheep cells but not by ox cells. After treatment with heated heterophilic antiserum, heterophilic antigen lost the ability to react with proper unheated serums, although the heated serums lack the ability to fix complement with a heterophilic antigen. The observation that normal rabbit serum has an inhibitory effect on the heterophilic antigenic extract similar to that of the heated heterophilic antiserum does not permit the attribution of a specific character to the phenomenon. Sachs concludes that the observation does not affect the unitary conception of the antibodies, but that it does not lend support to the other hypotheses.

I. DAVIDSOHN.

B ANTIGEN AND B ANTIBODIES IN MAN AND ANIMALS. V. FRIEDENREICH and S. WITH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:152, 1933.

Human anti-B agglutinins are absorbed by human red cells of type B and by rabbit red cells. The anti-B agglutinins of rabbit immune serum are not absorbed by rabbit red cells. The two B qualities and the two anti-B agglutinins are not identical. The human red cells of type B contain a B<sub>2</sub> quality, which they have in common with the rabbit red cells, and a B<sub>1</sub> quality, which is not shared. Thus the rabbit immune serum produced by injection of human B erythrocytes contains only anti-B<sub>1</sub> agglutinins. The titers of the anti-B<sub>1</sub> agglutinins in human serums vary considerably. The B quality was found in the red cells of the dog, rat, pig, and guinea-pig, but not in those of the ox, sheep, goat, chicken and pigeon. The B<sub>1</sub> quality was not found. Anti-B<sub>2</sub> agglutinins were found in the blood serum of chickens, while in all other species only anti-B<sub>1</sub> agglutinins were present.

I. DAVIDSOHN.

COMPARATIVE COMPLEMENT-FIXATION TESTS IN LEPROSY, TUBERCULOSIS AND SYPHILIS. G. KORNEL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:207, 1933.

The technic for the preparation of a leproma antigen is described. It was somewhat more specific than various syphilitic antigens and the antigen of Witebsky-Klingenstein-Kuhn, for which a marked specificity in the complement-fixation test for tuberculosis was claimed by its authors. The antigens employed for the serologic diagnosis of syphilis and of tuberculosis did not permit a differentiation of leprosy in the presence of simultaneous tuberculous or syphilitic infections.

I. DAVIDSOHN.

ANTIGENIC QUALITIES OF BACTERIA TREATED WITH ULTRAVIOLET RAYS. ST. GÄRTNER and J. SZATHMÁRY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:256, 1933.

Typhoid and paratyphoid B bacilli and *Vibrio aquatilis* were exposed for sixty minutes and one hundred and twenty minutes to ultraviolet radiation. Their ability to produce agglutinins in rabbits decreased progressively with the exposure; the loss of antigenic qualities was proportionate to the sensitiveness of the bacterial species to irradiation, which was measured by estimation of changes in the turbidity of the bacterial suspension. The specificity and the resistance to thermal and chemical influences were not influenced by irradiation.

I. DAVIDSOHN.

CAUSE OF THE POSITIVE WASSERMANN REACTION IN MALARIA. O. FISCHER and O. D. GÜNSBERGER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:295, 1933.

The frequency of a positive Wassermann reaction during, and for various lengths of time after, malarial attacks is due, according to Fischer and Günsberger, to the development of antibodies against the lipoids of the destroyed red blood cells. An alcoholic extract of erythrocytes reacted in twenty-one of twenty-eight cases with serums of patients with malaria, occasionally but only in low dilutions with syphilitic serums and not at all with normal Wassermann-negative serums. The differentiation between the serums of patients with malaria and with syphilis was possible by the evaluation of the titers obtained in complement-fixation tests with various extracts. A highly sensitive beef heart extract was particularly helpful.

I. DAVIDSOHN.

### Tumors

DIFFUSE CYSTADENOMA OF THE PANCREAS. G. C. PARENTI, *Pathologica* **25**:1, 1933.

A case of diffuse cystadenoma of the pancreas is reported. After a detailed description of the microscopic appearance of the tumor, the conclusion is reached that it originated from aberrant embryonic cell rests.

JACOB KLEIN.

CENTRAL CHONDROSARCOMA OF METAPHYSES. N. GULECKE, *Arch. f. klin. Chir.* **174**:401, 1933.

The first of the three cases of central chondrosarcoma of a tubular bone near the joint was noticed after fracture of the neck of the femur in a 48 year old woman. Symptoms existed seven years prior to the diagnosis, which was made by biopsy. When shortly afterward the patient died of hemorrhagic pancreatitis no metastases were found in the internal organs. The second tumor, in a 30 year old woman, was treated for some time as tuberculosis of the hip joint. Eight years after the first symptoms the involved portion of the femur was resected. Four and one-half years after the operation the patient died with a local recurrence of the tumor. The third tumor occurred in the humerus of a 58 year old bartender. Fourteen years after the earliest symptoms the upper half of the humerus was resected. Eleven years after the operation the patient was still alive, but had pulmonary metastases and a local recurrence.

The course of a tumor of this type is long and relatively benign as compared with that of a central sarcoma. Because metastases are late, operation should be undertaken even in late stages. The roentgenogram is usually so characteristic as to enable one to make the right diagnosis. In doubtful cases, biopsy is indicated.

C. ALEXANDER HELLWIG.

TWO CASES OF METASTASIZING NEURINOMAS OF THE GASTRO-INTESTINAL TRACT. KURT DENECKE, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:242, 1932.

The author gives a macroscopic and microscopic description of a pedunculated solitary neurinoma of the duodenum and of a cystic neurinoma of the stomach. In both cases large metastases to the liver were present. No glia fibrils and no axis-cylinders were found in the primary tumors and the metastases. From the histologic structure, the author concludes that these neurinomas originated from the cells of the sheath of Schwann.

C. ALEXANDER HELLWIG.

A CASE OF SO-CALLED CHONDROMA OF THE LUNG. RICHARD PETERS, *Beitr. z. path. Anat. u. z. allg. Path.* **89**:484, 1932.

In a 69 year old man a large chondroma was found at autopsy, located in the center of the lower lobe of the left lung. It was composed mostly of



cartilaginous tissue, but contained also mucous glands, unstriated muscle, fat and fibrous tissue. The complex tumor was derived from a misplaced anlage of a bronchus.

C. ALEXANDER HELLWIG.

PRODUCTION OF TUMOR-LIKE GROWTHS BY INORGANIC SALTS. ERNST LEUPOLD, Beitr. z. path. Anat. u. z. allg. Path. **89**:542, 1932.

Small papillomas were produced on the tongues of rabbits by injecting into the tissue hypotonic salt solutions. Simple washing of the oral cavity was also effective. The most potent substances were calcium chloride and sodium chloride. When the same substances were injected near artificial wounds in the ears of rabbits, fibroma-like growths appeared in the healing wounds. These tumor-like growths were not autonomous but disappeared spontaneously after cessation of the irritation. Disturbance in the salt concentration of the tissue is regarded by Leupold as an important factor in the etiology of neoplasms.

C. ALEXANDER HELLWIG.

A CASE OF MYOBLASTOMA ASSOCIATED WITH SQUAMOUS CELL CARCINOMA OF THE TONGUE. R. SCHIRMER, Beitr. z. path. Anat. u. z. allg. Path. **89**:613, 1932.

From the tongue of a 36 year old man a tumor was removed which was composed chiefly of very unripe myoblasts without striation. The epithelium covering the tumor had formed a ripe squamous cell carcinoma penetrating into the unripe rhabdomyoma. This myoblastoma is explained on the basis of Cohnheim's theory of misplaced tissue cells. In spite of their embryonal structure, the unripe rhabdomyomas are clinically benign, but they are frequently associated with atypical or cancerous proliferation of the covering epithelium.

C. ALEXANDER HELLWIG.

SUBLEUKEMIC RETICULO-ENDOTHELIOSIS TERMINATING IN RETICULO-ENDOTHELIAL SARCOMA OF THE HUMERUS. H. UNGAR, Beitr. z. path. Anat. u. z. allg. Path. **91**:59, 1933.

A woman, aged 48, whose illness had been characterized by pain in the sacroiliac region and progressive loss of weight and strength, died of lobar pneumonia seven months after the apparent onset of her illness. The first examination of the blood, two months after the onset, revealed a normal leukocyte count and distribution and anemia that led to a tentative diagnosis of pernicious anemia. In subsequent examinations the anemia, which progressed in spite of liver therapy, assumed the characters of a secondary anemia. The leukocyte count remained normal or was only slightly increased, but from 50 to 66 per cent of the cells were lymphocytic, leading to a diagnosis of aleukemic lymphadenosis. The spleen was palpable, but the superficial lymph nodes were not enlarged. At necropsy there was discovered a tumor of the humerus, apparently arising from the periosteum, with multiple metastases to the vertebrae, long bones and ribs. The reticulo-endothelium of the liver, spleen and bone marrow and of some of the lymph nodes was hyperplastic. In the liver and spleen transitions from reticulo-endothelial cells to lymphocytic cells like those of the peripheral blood could be seen. The condition is interpreted as a primary hyperplasia of the reticulo-endothelial system which terminated as a reticulo-endothelial sarcoma of bone. The reticulo-endothelial hyperplasia of the bone marrow is considered the intermediate stage between the generalized reticulo-endothelial hyperplasia and the terminal bone tumor. The case is made the basis of a discussion of the origin of the blood cells, the author adhering to the triplistic doctrine, although admitting that the active mesenchyme may give rise to the various types of blood cells under abnormal conditions. The relations of this case to other reported examples of reticulo-endothelial hyperplasia are discussed.

O. T. SCHULTZ,

HISTOLOGY OF MIXED TUMORS OF THE SALIVARY GLANDS. W. E. ZYMBAL, Beitr. z. path. Anat. u. z. allg. Path. **91**:113, 1933.

Zymbal's presentation of the histology and cytology of the so-called mixed tumors of the salivary glands is based on the study of fifty-five such tumors of the parotid gland, two of the submaxillary gland and one of the upper lip. Transitions from normal glandular tissue to tumor tissue and from one type of tumor tissue to another were observed. The wide variations in the cellular composition of these tumors, extending from tubular glandular structures of practically normal morphology to solid, carcinoma-like areas, are described and illustrated in detail. The wide variation in the structure of the mitochondrial apparatus of the cells is apparently dependent on differences in the secretory activity of the cells. The metachromatic ground substance of the tumors is the result chiefly of the secretory activity of the cells. The cartilage-like ground substance is derived from degenerated tumor cells, which contain variable amounts of mucus or mucoid material that enters into the formation of the cartilage-like material. In eight of thirteen tumors in which explantation of the fresh tissue was done, the preparations remained viable and exhibited evidences of growth for as long as three weeks, but no proliferating transplantable tissue cultures were obtained. The cellular character of the growing explant is dependent on the cellular character of the tumor tissue that is explanted. The author concludes that the tumors under discussion are purely epithelial in origin and should not be termed mixed tumors.

O. T. SCHULTZ.

EXPERIMENTALLY INDUCED EPITHELIAL PROLIFERATION OF THE RABBIT'S TONGUE. W. RONDORF, Beitr. z. path. Anat. u. z. allg. Path. **91**:176, 1933.

This is a contribution to the conceptions of Leupold, under whose direction the work was done, of the effect of local cellular metabolism, as influenced by certain organic and inorganic substances, on cellular proliferation. The substances used in these experiments were calcium chloride and potassium chloride in solutions of 1:1,000,000 strength, and leucine, glycogen and cholesterol, mostly in solutions of 1:1,000,000 and 1:10,000,000. The quantity injected just beneath the epithelium of the margin of the rabbit's tongue was 0.05 cc. This quantity and the strengths of the solutions are reminiscent of the homeopathic procedure of "proving" of drugs. The injections were repeated at intervals of from two to eight or ten days. Judged by the size and number of papillomas that developed, calcium chloride, leucine, glycogen and cholesterol were most effective; then came potassium chloride and cholesterol plus one or another of the salts, and least effective were water and cholesterol in concentrations of from 1:100,000 to 1:1,000. The findings are discussed in the light of Leupold's speculations.

O. T. SCHULTZ.

MULTIPLE ADENOMAS IN AN UNDESCENDED TESTIS. N. IDE, Beitr. z. path. Anat. u. z. allg. Path. **91**:241, 1933.

Exploratory laparotomy in a man, aged 48, who complained of gastro-intestinal symptoms revealed multiple metastases in the omentum and mesentery. The primary tumor was not discovered. It was known that the man had an undescended right testis. Microscopic examination of one of the metastases led to the diagnosis of carcinoma probably originating in the gastro-intestinal tract. At necropsy some months later the primary tumor was found to be in the pancreas. The undescended testis, which was situated in the inguinal canal, was the size of an olive. On microscopic examination it was found to be atrophic but to contain multiple tubular benign adenomas of the type originally described by Pick.

O. T. SCHULTZ.

CONGENITAL NEUROFIBROMATOUS MACROGLOSSIA. H. MÜLLER, Centralbl. f. allg. Path. u. path. Anat. **57**:55, 1933.

This rare condition was found in the body of a boy 6 years old who was mentally retarded. Over the entire body there were numerous nevi, some finely

and some coarsely pigmented. There was also a tumor of the right side of the dorsal third of the tongue. In the tongue the papillae were enlarged, and scattered in the muscle were pinhead-sized nodules and strands of gray-yellow tissue. The nodules contained medullated nerves in their central parts but no ganglion cells. The muscle about them was loose.

GEORGE RUKSTINAT.

EPITHELIAL CYST OF THE HEART. A. KOLATSCHOW, *Centralbl. f. allg. Path. u. path. Anat.* **57**:311, 1933.

A smooth-topped cyst 21 by 18 by 17 mm. was found at autopsy on the inner part of the wall of the left ventricle of the heart of a man 45 years old. About four fifths of it projected above the endocardium. The content was jelly-like. The cyst wall was fibrous, lay near the muscle and had no inflammation about it; the lining was of cylindric epithelium, ciliated in some regions. The author found no reports of similar cysts in the literature. He believes that this occurrence is best explained as dystopia. Another possible origin would be from the myo-epicardial plate if the possibility of metaplasia of epicardial mesothelium into cylindric epithelium were granted. About ten years prior to his death the patient had had an echinococcic cyst removed from the liver, and during the operation the cyst broke, spilling its contents into the abdomen. The author sees no connection between the liver and a cardiac cyst.

GEORGE RUKSTINAT.

METASTASIZING GANGLIONEUROMA OF THE SYMPATHETIC SYSTEM. D. HEINRICI, *Centralbl. f. allg. Path. u. path. Anat.* **58**:1, 1933.

At the necropsy on a man 32 years old, who had an illness clinically resembling a disorder of the hematopoietic system, a tumor of the left abdominal sympathetic ganglion was found. The growth had two distinct portions, one of normal-appearing nerves and connective tissue and another of small round cells poor in cytoplasm. The latter cells occurred also in metastases in the ribs, vertebrae, liver and lumbar and peripancreatic lymph glands.

GEORGE RUKSTINAT.

SIGNIFICANCE OF THE SPLEEN IN THE GROWTH AND METABOLISM OF MALIGNANT TUMORS. W. BÜNGELER, *Frankfurt. Ztschr. f. Path.* **43**:409, 1932.

That the spleen may play an important inhibitory rôle in the development of tumors is suggested by the rarity of its involvement in such growths. From experiments with mice into which tumors were transplanted the author concludes that the spleen has an inhibitory effect on malignant growth. Extracts of splenic tissue inhibit fermentation and increase respiration of tumor cells but also, to a lesser degree, inhibit fermentation and increase respiration of normal cells. The inhibitory agent is water-soluble, is not bound to proteins or lipoids and is not destroyed by heat (100 C.). The effects described are not confined to extracts of spleens but can be produced by extracts of other organs. These extracts, however, are less efficient.

O. SAPHIR.

DO GIANT CELL SÂRCOMAS EXIST? E. KOTZIAN, *Frankfurt. Ztschr. f. Path.* **43**:484, 1932.

The author reviews reported cases of giant cell tumors. While many such tumors are not malignant, not even being tumors in the sense of a neoplasm, the occurrence of true giant cell sarcoma cannot be denied. The author also discusses the various possible sources of the giant cells.

O. SAPHIR.

PRIMARY PLASMOCYTOMA. G. W. MOLOTKOFF, *Frankfurt. Ztschr. f. Path.* **43**:508, 1932.

Two cases are reported. In the first, the tumor was located in the submucosa of the appendix. It consisted almost exclusively of plasma cells with marked

pyroninophilic cytoplasm and eccentrically situated nuclei. Mitotic figures were present. A fine network of collagenous fibers was recognizable, sometimes in close relation to the cells. Occasionally giant cells were present. The tumor had invaded the muscularis and the mucosa. The author believes that this is the first instance of plasmocytoma of the appendix described in the literature. In the second instance the tumor was found in the neck of a 6 year old boy. The architecture of the involved nodes was obscured. There was a marked proliferation of plasma cells similar to those in the first case. Much reticulum was present. Occasionally giant cells with basophilic cytoplasm were found. The belief is expressed that in both cases the condition was a localized plasma cell lymphogranulomatosis.

O. SAPHIR.

EXTENSIVE BLASTOMATOSIS OF THE LYMPHOHEMATOPOIETIC APPARATUS. R. GLAUNER, Frankfurt. *Ztschr. f. Path.* 44:105, 1932.

A 50 year old man had an enlargement of the axillary, inguinal and supra-clavicular lymph nodes. One of the latter was removed and revealed a solid carcinoma. The patient died six months later, and autopsy disclosed small grayish-white foci in the pectoral, abdominal, psoas and iliac muscles and on the peritoneal surface. There were large, firm, white masses in the region of the lymph nodes corresponding to the bifurcation of the trachea. Small whitish nodules were found in the epicardium, the lungs, the spleen, the liver and the mucosa of the stomach and gastro-intestinal tract. The hepatogastric and hepatoduodenal ligaments also were diffusely infiltrated by whitish tumor masses. Tumor nodules were found in the cerebellum. The bone marrow corresponding to the right femur revealed large whitish areas surrounded by dark red bone marrow. The gross diagnosis was "disseminated blastomatosis." A primary tumor could not be found. Histologically the tumor nodes revealed a fine reticular and connective tissue network in the meshes of which there were round, oval and polygonal cells with large, well outlined nuclei showing fine chromatin network and distinct nucleoli. The cells were often well separated. Occasionally, however, they formed a syncytium. Sometimes giant cells, pyknotic nuclei and mitoses were present. Phagocytosis was frequently noted. Many cells contained lipid granules. Occasionally nests of tumor cells were found in the lymphatics. The hypophysis revealed an adenoma consisting of chief cells. Because of the dividing of the tumor in organs belonging to the reticulo-endothelial system and because of the reticular structure of the tumor nodes, the marked phagocytosis of the cells and their ability to store fat, it is possible that this case is one of generalized disease of the reticulo-endothelial system (reticulosis). On the other hand, the fact that before death a node was removed which histologically revealed carcinoma and the fact that nests of tumor cells were found in lymphatics are in favor of the view that the nodules were metastases of a carcinoma, the primary carcinoma not having been found. The tumor formation might also be explained as a reaction of the reticulo-endothelial system to irritation either by the metastatic carcinoma cells or by their poisonous products.

O. SAPHIR.

COMBINATION TUMOR OF THE BRAIN (TRUE ANGIOGLIOMA). A. WEISS, Frankfurt. *Ztschr. f. Path.* 44:144, 1932.

A tumor in the brain revealed structures of a cavernous hemangioma with true bone formation within a hyalinized stroma and those of a glioma which surrounded the hemangiomatous structures. The author believes that the hemangioma was the primary tumor, arising from a malformed anlage of the tuberculum intercolumnare. The anatomy and histology of this structure are discussed. It was first described by Putnam (*Bull. Johns Hopkins Hosp.* 33:181, 1922). The opinion is expressed that the primary hemangioma caused a secondary glial reaction, which in turn formed the basis for the glioma. There also must have been a hereditary predisposition; otherwise a simple proliferation of glia would not have developed into a glioma.

O. SAPHIR.

FUNDAMENTAL QUESTIONS IN TUMOR RESEARCH. B. FISCHER-WASELS, Frankfurt. *Ztschr. f. Path.* **44**:177, 1932.

On the basis of the results of researches performed by himself and his assistants, the author refutes the theory that tumors have their origin in irritation of tissues, not because an irritation cannot be demonstrated but because too many factors are present all of which can be interpreted as irritation. Every factor must be analyzed separately and should not be disregarded or carelessly brought in consideration under the broad term of irritation. He does not believe that trauma alone is an important factor, and he contradicts a statement in the literature that a large number of tumors of the brain are caused by trauma resulting in primary areas of necrosis of the brain which produce reactive gliosis, which in turn gives rise to malignant growth. He maintains the importance of the "embryonal tumor anlage" and also of the hereditary factor. He summarizes a number of basic principles in regard to the formation of tumors, namely, "the law of primary local tissue insults," "the law of the typical latent period," which may vary from four to fifty-five years, "the law of the formation of a primary tumor anlage" and "the law of the sensible period of tumor formation." He also emphasizes the importance of regeneration in the formation of tumors. As to the question of "a special tumor constitution," he refers to experiments in animals which received tar for a long period. In these animals wounds as the result of burns did not heal with the formation of scars but underwent tumor formation. He concludes that tar and similar chemicals alter the constitution so that an irritant which under normal conditions results in a wound and scar may produce a tumor. A "tumor constitution" is produced also in producing alkalosis of the blood which is supposedly typical of cancer. The morphologic appearance of tumor cells, the marked irregularities of the nucleus and of the chromatin structures, and the many irregular mitoses make it seem possible that the active principles of the tumor lie in a changed nuclear substance of the tumor cells. Systematic examinations of the chemistry of the nuclear substances and the study of their metabolism by the use of the new methods of nuclein metabolism may offer a rich field for further investigation.

O. SAPHIR.

MULTIPLE CAVERNOMA OF THE PLEURA. G. MARTENS, Frankfurt. *Ztschr. f. Path.* **44**:272, 1932.

Multiple cavernoma was found in the pleura of a 56 year old man who died as the result of hemothorax. Microscopically the growths were true tumors. It is therefore not likely that they were remnants of healed pleuritis. The author discusses the relation between the hemorrhage into the chest and cirrhosis of the liver, which was also present.

O. SAPHIR.

TUMORS OF THE CHOROID PLEXUS, EPENDYMOMAS AND NEURO-EPITHELIOMAS. H. URBAN, Frankfurt. *Ztschr. f. Path.* **44**:277, 1932.

These tumors cannot be differentiated clinically, but they are distinguishable histologically. A hematoxylin-eosin-safranin method for studying their morphologic appearance is recommended.

AUTHOR'S SUMMARY.

SYSTEMIC RETICULO-ENDOTHELIAL HYPERPLASIA WITH TUMOR-LIKE FORMATIONS IN A CASE OF CHRONIC LYMPHATIC LEUKEMIA. J. LOESCH, Frankfurt. *Ztschr. f. Path.* **44**:351, 1932.

A 47 year old patient received antisyphilitic treatment for several years. About one year after the last treatment he noticed swelling of the axillary lymph nodes. He was admitted to the hospital with a diagnosis of chronic lymphatic leukemia. Autopsy revealed changes characteristic of lymphatic leukemia and also whitish nodes in the liver and spleen. Microscopically, a diffuse proliferation of reticulo-endothelial cells was found in the spleen, liver and bone marrow. In the spleen

and liver this assumed the form of distinct nodules. Some of the lymph nodes showed changes characteristic of lymphatic leukemia, while others revealed marked proliferation of reticulum cells, which in some instances had completely replaced the lymphatic cells. Transitions from lymphatic cells to reticulum cells, however, could not be found.

AUTHOR'S SUMMARY.

### Medicolegal Pathology

SUDDEN DEATH AND SYPHILIS. A. LEE BRISKMANN, *Am. J. Syph.* **16**:471, 1932.

Syphilitic disease is assigned as the cause in 30 per cent of twenty-four cases of sudden death from coronary sclerosis.

INJURY OF THE MEDULLA IN PUNCTURE OF THE CISTERNA MAGNA. A. R. VONDERAHE and F. C. HABERMAN, *Arch. Neurol. & Psychiat.* **29**:166, 1933.

A cisternal puncture was made in a patient for the diagnosis of meningitis. A second puncture was unsuccessful, and the patient died within four hours. Autopsy revealed two needle puncture wounds in the medullary portion of the brain stem with a surrounding hemorrhage that extended into the fourth ventricle.

WILLIAM FREEMAN.

CORONARY SCLEROSIS: AS SEEN IN A CORONER'S OFFICE. A. A. BERGER, *California & West. Med.* **36**:332, 1932.

The author analyzes statistically the causes of death in 4,754 autopsies that came to his attention from 1928 to 1930 inclusive. Approximately 50 per cent of the deaths were due to "natural causes" (nonintentional and nontraumatic). Approximately 8 per cent of all deaths due to natural causes (4 per cent of the total number of deaths) were due entirely to coronary disease in one form or another (ruptured heart, ruptured coronary aneurysms, coronary sclerosis with occlusion, coronary thrombosis). Coronary disease was found in 50 per cent of the remaining cases of death due to natural causes.

WILLIAM FREEMAN.

DEATH FOLLOWING INGESTION OF FIVE GRAINS OF ACETYLSALICYLIC ACID. B. R. DYSART, *J. A. M. A.* **101**:446, 1933.

A 45 year old white woman, unmarried, a teacher of art, who for fifteen years had suffered with increasing severity from "asthma" following an automobile accident, had taken epinephrine hypodermically for the terminal three years with only occasional relief. Allergically she was also sensitive to many foods. One day she took one 5 grain tablet of acetylsalicylic acid, and one minute later she went through an "asthmatic" attack and died before a physician could be called, the interval being probably about ten minutes. The postmortem examination by the coroner revealed only some tenacious mucus in the bronchi and a sarcoma of the dura about the size of an English walnut located over the left cerebral hemisphere.

WILLIAM FREEMAN.

BLOOD GROUPING IN QUESTIONS OF PARENTAGE. A. L. WIENER, *J. Immunol.* **24**:443, 1933; *Am. J. M. Sc.* **186**:257, 1933.

The chances of proving nonpaternity when the blood type of the falsely accused man is known were calculated. In New York City the average chances of proving nonpaternity by means of all four agglutinogens, A, B, M and N, are approximately one in three. A man of type A++ has less than one chance in ten to prove his innocence, and a man of type B++ has only one chance in seven. Men of the remaining ten types, however, have chances ranging from one in four

(O++) to two in three (AB—+). The chances of detecting interchange of infants in hospitals by means of the agglutinogens A, B, M and N are seven in ten.

The medicolegal application of blood grouping for the determination of non-paternity is urged on the basis of the experiences of European countries during the past ten years. The application of the agglutinogens M and N of Landsteiner and Levine is also fully justified at present on the basis of studies of families including more than 3,000 children. The method of application is illustrated by actual experiences in 6 cases, in 3 of which valuable information was obtained.

AUTHOR'S SUMMARIES.

TRAUMATIC RUPTURE OF THE PANCREAS. H. K. RANSOM, J. Michigan M. Soc. **31:332**, 1932.

The author reports a case of uncomplicated complete vertical rupture of the pancreas at the junction of the head and body in a 28 year old man who received severe abdominal injuries in a street fight.

WILLIAM FREEMAN.

SYMPOSIUM ON THE FORENSIC VALUE OF BLOOD GROUPING. M. Times **60:203**, 1932.

Attention is called to this symposium, in which George I. Swetlow discusses the legal applications of blood grouping; S. H. Polayes, the principles and the technic of determining the blood groups; A. S. Wiener, the theory of blood grouping with special reference to heredity, and Max Lederer, the practical application of blood grouping. The question of admitting the results of tests for blood grouping in courts in this country is discussed.

CHANGES IN THE SPLEEN AND MARROW IN ACUTE CARBON MONOXIDE POISONING. F. WIETHOLD, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21:325**, 1933.

In a series of 200 fatal cases of acute carbon monoxide poisoning the gas was demonstrated in the blood of the spleen and the bone marrow. Increased hemato-poiesis and vascular dilatation were observed in the marrow.

JACOB KLEIN.

PROTRACTED RETENTION OF ALCOHOL IN TRAUMATIC COMA. R. M. MAYER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21:337**, 1933.

Alcohol previously imbibed may be found in the urinary bladder for a considerable time after death. In the author's case alcohol was demonstrated in the blood thirty hours after the onset of coma. Traumatic coma delays the excretion of alcohol.

JACOB KLEIN.

EXPERIMENTAL STUDIES ON POISONING BY INDUSTRIAL ARSENIURETED HYDROGEN. G. SCHRADER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21:342**, 1933.

Observation of a fatal case of arsine poisoning in the industrial preparation of cadmium led to a more detailed histologic and experimental study. The outstanding lesions were hemolysis and degenerative changes in the kidneys, liver and heart. Experiments on guinea-pigs revealed similar changes, particularly in the liver and heart. It is of medicolegal interest to note that in some cases permanent cardiac damage may result.

JACOB KLEIN.

RUPTURE OF THE DUCTUS ARTERIOSUS. E. FRITZ, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21:365**, 1933.

After an accident a woman gave birth to a fetus 43 cm. long which died four and one-half hours later. At autopsy on the fetus three small perforations of the

ductus Botalli were found. Microscopically these three areas showed complete tears with hemorrhage between the layers of the vessel wall and the adventitia. In explanation of this rare occurrence it is pointed out that hemorrhages of the vasa vasorum occur frequently in the ductus botalli.

JACOB KLEIN.

FORENSIC SIGNIFICANCE OF BLOOD TYPES M AND N AND OTHER TYPES.  
F. SCHIFF, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **21**:404, 1933.

There are other blood types besides those indicated in the usual four group classification. By suitable tests with immune serum M and N types have been demonstrated. Moreover, group A of the ordinary classification is found to consist of two groups, A<sub>1</sub> and A<sub>2</sub>. There is a P factor, as well as the factors excreted in the saliva. M and N are constant in their occurrence and follow the mendelian law. They are demonstrated even in the blood of the new-born. In medicolegal practice these groups are of value in studying cases of doubtful paternity, illegitimacy and exchange of infants, and even in identifying persons. Thus in a family of eight it was possible to distinguish between all but two by means of blood grouping.

JACOB KLEIN.

### Technical

RELATION BETWEEN THE CEREBROSPINAL FLUID AND THE BLOOD SERUM IN SYPHILIS. F. E. HAAG and H. KOLBE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:380, 1932.

The extract of syphilitic liver, the citochol extract and the Bordet extracts were employed as antigens in the complement-fixation tests for blood serum and cerebrospinal fluid. The serums were also tested with three precipitation tests: the Meinicke, the citochol-Kahn and the Mueller *Ballung* reaction II. The cerebrospinal fluid was tested with the Lange colloidal gold test. In 1,400 cases, comparative tests of the blood serum and the cerebrospinal fluid were carried out. The precipitation tests were always specific; examination of the blood serum, particularly with precipitation tests, is essential for establishing the specificity of the results in examination of the cerebrospinal fluid, particularly when the latter shows a positive colloidal gold test with a negative complement-fixation reaction, as is frequently seen in multiple sclerosis. Positive signs in the cerebrospinal fluid with negative signs in the blood serum were occasionally encountered in persons with old treated syphilis of the central nervous system. Since the employment of the sensitive precipitation tests for the examination of the blood serum, the number of discrepant observations on the blood and cerebrospinal fluid has greatly diminished.

I. DAVIDSOHN.

DETERMINATION OF THE TITER OF ISO-AGGLUTININS. JORMA PIKKARAINEN and Y. K. SUOMINEN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:145, 1933.

The use of pipets (length, 30 cm.; volume, 0.1 cc.) for determination of the iso-agglutinin titer permitted a greater accuracy and uniformity than the use of test tubes, in a series of a hundred comparative tests.

I. DAVIDSOHN.

QUICK DIAGNOSIS IN EPIDEMIC CEREBROSPINAL MENINGITIS. S. I. GINSBURG, W. S. KALININ and A. P. GULJAWEA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:463, 1933.

A complement-fixation test was developed by Ginsburg and his associates, employing as an antigen cerebrospinal fluid heated at 55 C. for thirty minutes. Polyvalent antimeningococcus serum served as an antibody. The performance of the test takes from three to four hours. Of 252 clinically established cases, 176 (70 per cent) were serologically, and 137 (54 per cent) bacteriologically, positive. In only 2 bacteriologically positive cases was the serologic test negative. In the



control series of cerebrospinal fluids, including those of persons with tuberculous and pneumococcic meningitis or cerebrospinal syphilis and normal persons, the test was negative. Control tests with the cerebrospinal fluid of patients having meningococcic meningitis were negative with normal horse serum and with anti-pneumococcus serum, while weak positive results were obtained when antigenococcus serum was used. Precipitation tests with cerebrospinal fluid and specific anti-meningococcus serum showed a much lower degree of sensitivity than the complement-fixation tests. The positive reaction became negative after termination of the disease.

I. DAVIDSOHN.

COMPARISON OF THE RESULTS OF THE SACHS AND GEORGI (LENTOCHOL) REACTION AFTER TWENTY-FOUR AND FORTY-EIGHT HOURS. HEINZ ECKARDT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:497, 1933.

The test tubes were kept in the incubator for the first twenty-four hours and at room temperature for the second twenty-four hours. In 496 serums, or 6.5 per cent of the definitely syphilitic ones, only the second reading, after forty-eight hours, was positive. In 275 of the syphilitic serums these reactions were the only positive results, because the serums showed a negative Wassermann reaction. Nonspecific positive results were not observed. The results after forty-eight hours showed an increased sensitivity in cases of early syphilis, during provocative and during regular treatment. The inclusion of the reading after forty-eight hours raises the percentage of agreements with the Wassermann reaction to 96.3.

I. DAVIDSOHN.

PRECIPITATION TESTS FOR THE SEROLOGIC DIAGNOSIS OF SYPHILIS. Z. MILIŃSKA-SZWOJNICKA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:139, 1933.

The citochol reaction I and II of Sachs-Witebsky, the "ball" reaction of Mueller and the Meinicke *Klärung* reaction were compared with the Bordet-Wassermann complement-fixation test, 27,273 clinically checked syphilitic serums and cerebrospinal fluids and 2,563 negative control serums being used. The citochol reactions showed the highest degree of sensitiveness and of specificity; the Meinicke test proved least satisfactory. Milińska-Szwojnicka warns against the replacement of the complement-fixation test with a precipitation test. Both should be employed simultaneously.

I. DAVIDSOHN.

THE PHYSICAL FITNESS OF RABBITS FOR THE PRODUCTION OF PRECIPITATING ANTISERUMS. P. VON GARA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:171, 1933.

A total of 230 rabbits were employed for the production of precipitating anti-serums in the Hygiene Institute of the University of Greifswald, which is one of the official serum institutes in Germany. The immunization was carried out according to Uhlenhuth. One cubic centimeter of serum, preferably fresh, was injected on the first, fourth and seventh days. Additional injections followed if the titer was less than 1:20,000. Not more than 15 per cent of the animals gave serums which were satisfactory in every respect, showing the necessity of using at least six animals in order to obtain a good specific precipitating serum. The best results were obtained with male white rabbits weighing over 2,500 Gm. During the summer, the serums showed fewer nonspecific heterologous precipitins.

I. DAVIDSOHN.

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

E. H. HATTON, *President, in the Chair*

*Regular Monthly Meeting, Dec. 11, 1933*

### THE ISO-ANTIGENIC PROPERTIES OF CASEIN. JULIAN H. LEWIS.

Casein is one of the several antigens of animal origin which lack species specificity; such an antigen from one species reacts in common with the same kind of antigen from other species. Chemically and immunologically casein from one source is identical with caseins from all other sources. Antigens which are indistinguishable immunologically should serve as antigen in the species from which they are obtained just as efficaciously as they do in foreign species. The iso-antigenic properties of crystalline lens, brain and testicle had been proved, but no test had, as yet, been made on casein.

A lactating goat was given sixteen intravenous injections of 1 Gm. of casein prepared from her own milk and dissolved at  $p_H$  7. Another lactating goat was given similar injections of casein from cow's milk. The immune serums from these two goats, as well as the normal serums taken before the injections, were tested by complement fixation with caseins from cow's and from goat's milk. Both immune serums reacted equally with both types of caseins. This experimental proof of iso-antigenicity is also supported by clinical evidence. A lactating mother had allergic reactions when she weaned her child and attempted to stop the mammary secretion. Cutaneous tests made with her own milk gave severe reactions. Since the proteins of whey show strong species specificity and are incapable of exciting the formation of antibodies in the homologous species, it is probable that the active antigen in this mother was the casein.

#### DISCUSSION

H. C. SWEANY: What part does heredity play in allergy?

J. H. LEWIS: Heredity has a rôle in allergic diseases, but statistics vary as to its importance. A man subject to severe attacks of hay fever reared two children, one adopted, the other his own, under identical conditions. Hay fever developed in the son at 20 years of age but not in the adopted son.

### SO-CALLED ALVEOLAR CELL CARCINOMA OF THE LUNG. H. C. SWEANY.

A uniformly low columnar cell carcinoma of the lung spread directly from one alveolus to another until all of the right lung and the middle of the left were consolidated masses with an ashen-gray appearance. The cells first supplanted the normal alveoli and maintained an alveolar arrangement. Some formed papillary growths into the alveoli. In the older region the cells disintegrated. Because of the low columnar type of cells the growth seems to have had its origin somewhere in the lower bronchi or bronchioles. There has never been proof that carcinoma of the lung has originated from the pulmonary alveolar cells. Probably the question can be answered only when the precursor of the alveolar cell is established.

#### DISCUSSION

V. LEVINE: I have seen several instances of carcinoma in the periphery of the lung. In some of the cases the growth originated in a dilated bronchus near the pleura. Thus, a carcinoma limited to the surface of the lung does not necessarily arise from alveolar epithelium.

## CYTOLOGY OF NASAL POLYPS. THEODORE E. WALSH.

Interest has long been shown in the etiology of nasal polyps because of the differences in the clinical findings in the patients in whom they occur. Polyps occur in the nose as the result of a marked acute or chronic inflammation. However, it is not generally agreed that infection is necessarily the cause of the inflammation.

Clinically there are two groups of patients with nasal polyps: first, those in whom there is definite evidence of infection, as indicated by the presence of purulent secretion in the nose, and second, those in whom there is no exudate but rather a profuse watery nasal secretion, i. e., those with so-called vasomotor rhinitis. It has long been recognized by the more conservative observers that vasomotor rhinitis is not a surgical condition and that intranasal operative procedures in an uncomplicated case will not effect a cure, whereas when the inflammatory condition is due to sensitivity to some allergen, removal of the offending allergen will usually alleviate the symptoms and often effect a cure. On the other hand, a patient suffering from vasomotor rhinitis is usually subject to intranasal infection, and when infection is a complicating factor, operative interference is frequently a necessity. In the case of polyps, it is of value to know whether they are due to infection alone or are caused primarily by the vasomotor rhinitis, because on this knowledge depend the course of treatment adopted and the prognosis.

Some recent observations on the cellular components of nasal secretions in various cases of rhinitis led to the conclusion that in vasomotor rhinitis, whether allergy is demonstrable or not, the nasal secretions contain a high percentage of eosinophil leukocytes, whereas in infectious rhinitis there are never more than 5 per cent of eosinophil leukocytes in the secretions. This cytologic study was continued to include polyps in order to ascertain whether by correlation of the clinical findings and the cytology of the polyps any definite conclusion in regard to their etiology could be reached.

The polyps were fixed in a solution of formaldehyde or in Zenker's fluid to which a dilute solution of formaldehyde had been added, embedded in paraffin and cut at 10 microns. They were stained with Maximow's hematoxylin eosin azure stain. The cells were present in greatest number immediately beneath the basement membrane, while deeper in the tissue they were sparsely scattered. There appeared to be two distinct types of polyps—those with many eosinophil leukocytes and those with only occasional ones. In all the polyps examined there were numbers of mononuclear cells and many plasma cells. Polymorphonuclear neutrophils were seen in the polyps from persons who had clinical signs of infection. Of sixty persons whose polyps were examined, thirty-seven had polyps showing many eosinophil cells and twenty-three had polyps showing only an occasional eosinophil cell. Complete allergic investigations were made in nineteen of the thirty-seven persons whose polyps contained many eosinophil leukocytes and intradermal tests with one or more allergens were positive in fourteen and not in five of them. Of these five persons four had definitely low blood pressure and low rates of basal metabolism and one had marked hypertension. Of the twenty-three persons with few eosinophil leukocytes in the polyps, only two had complete allergic investigation and neither had any positive intradermal test or abnormality in blood pressure or metabolism. Of the thirty-seven persons with many eosinophil leukocytes in the polyps, fourteen had and twenty-three did not have clinical evidence of infection as shown by the presence or the complete absence of nasal exudate. However, of the twenty-three with few eosinophil leukocytes in the sections all had a purulent nasal discharge. In ten of these the pathologic conditions in the nose were unilateral and in thirteen bilateral, while in the thirty-seven who had many eosinophil leukocytes in the polyps, the pathologic changes were unilateral in only one and bilateral in thirty-six.

Diametrically opposite views on the etiology of nasal polyps have recently appeared in the literature; it is suggested on the one hand that allergy is to be

suspected in all cases of nasal polyps, and on the other that nasal polyps are always due to infection. The findings in the series of sixty cases reported here led to the following conclusions:

1. There are two types of nasal polyps, (a) those with many eosinophil leukocytes and (b) those with few.
2. Polyps with an abundance of eosinophil cells are due to the condition known as vasomotor rhinitis, which may be allergic.
3. Polyps with few eosinophil leukocytes are due to infection alone.

#### VASCULAR CHANGES IN CHRONIC EXPERIMENTAL ATELECTASIS. W. E. ADAMS.

The volume of the flow of blood in a normal lung and its variations under abnormal inflation have held the interest and attention of investigators in this field since the middle of the nineteenth century. Although extensive studies have been carried out by many investigators, no definite conclusion has been reached. The vascular changes occurring in an atelectatic lung during periods varying from a few days to over one and one-half years were studied.

Massive atelectasis of one lung was produced in forty-five dogs by completely occluding the bronchus to that lung by the technic in which nitrate of silver is used (*Ann. Surg.* 95:106, 1932). The animals were killed at intervals of from a few days to one and one-half years. India ink was injected into the blood vessels in a portion of the atelectatic lung in some of the animals, according to the technic of Coryllos and Birnbaum (*Arch. Surg.* 19:1346, 1929). In order to obtain microscopic sections of atelectatic tissue in the same lung before and after the injection of india ink, a portion of the lung was ligated through a thoracotomic opening before the injection. In another group of animals, reinflation of the atelectatic tissue was accomplished through a thoracotomic opening by injecting air through a needle inserted into the bronchus distal to the region of stenosis. Reinflation was obtained at a pressure of between 35 and 40 mm. of mercury. Again a portion of the reinflated tissue was tied off before the injection of india ink. Thus microscopic sections of reinflated atelectatic tissue before and after the injection of ink were made available.

Microscopic sections of pulmonary tumors that had been atelectatic for a few days revealed dilated and tortuous capillaries readily filled with india ink. The capillaries were so numerous that the tissue resembled hemangioma. Atelectatic lobes at this stage could be reinflated to almost their original size. By the end of one month the number and size of the capillaries in the atelectatic tissue had decreased. There were no other changes. By the end of four months there were large spaces filled with blood, chiefly at the periphery of the parenchyma of the lung. These appeared to be alveoli containing blood. At this stage a considerable amount of fragmentation and desquamation of bronchial and alveolar epithelium was noted. From the fourth until the fifteenth month there was a gradual increase in the size and number of the blood spaces with a decrease in the number of alveolar walls. A gradual increase in the amount of tissue with changes was also observed. Not infrequently there was blood in the smaller bronchioles. Following the injection of india ink there appeared to be a partial washing out of the blood from the spaces, but at no time was ink observed within them. Reinflation of atelectatic tissue during this period caused a moderate tearing of the alveoli. The introduced air entered only a portion of the spaces filled with blood. There appeared to be less blood removed from the spaces following reinflation of the atelectatic tissue.

From these observations it is difficult to understand how the blood in the spaces could have been supplied by the pulmonary circulation. The lack of blood pigment in any of the microscopic sections precludes the possibility that the blood in the spaces was a hemorrhage into the pulmonary parenchyma. That some of the spaces contained less blood following the injection of india ink, together with the lack of blood pigment, strongly suggests that this blood was in the circulation. Possibly the spaces filled with blood were in direct connection with the bronchial

arterial system and the pressure in this system may have been sufficiently great to break through an atrophied and degenerated wall between the original alveoli and the circulating blood. Evidence of degeneration and fragmentation of the epithelium of both the bronchioles and the alveolar walls lends support to this view. If this is true, injection through the pulmonary system would be less likely to replace the blood in such spaces with india ink. This question is being investigated.

#### UNUSUAL PEPTIC ULCERS OF THE GREATER CURVATURE OF THE STOMACH. WARREN B. MATTHEWS.

A woman aged 57 had had symptoms of ulcer without complications for thirty-five years. Roentgen examination demonstrated a filling defect of the most dependent portion of the greater curvature of the stomach. No free acidity was obtained in an Ewald test meal. Two thirds of the stomach was resected by Dr. Phemister. The patient has been followed for five and one-half years since the operation. She has remained in good health. The excised tissues contained an ulcer 1.5 cm. in diameter astride the greater curvature 12 cm. from the pylorus. The ulcer had perforated only to the muscularis. Microscopically it was a typical chronic peptic ulcer, without malignant changes.

A man aged 56 had suffered epigastric pain for one year. In spite of a loss in weight of 20 pounds (9 Kg.) he seemed in fair health. Roentgen examination revealed a large crater in the middle third of the greater curvature of the stomach. An injection of histamine stimulated a gastric secretion with a free acidity of 52 clinical units. The preoperative diagnosis was: carcinoma. Dr. Phemister removed two thirds of the stomach because of a lesion on the greater curvature, which was adherent to the transverse colon. The patient has been well for fifteen months since the operation. The excised mass had an ulcer overriding the greater curvature, 15 cm. from the pylorus, which had perforated all the layers of the wall of the stomach and had a base of thickened, indurated omentum. Microscopically the lesion was a scarred peptic ulcer considerably infiltrated at the base by round and plasma cells and eosinophil leukocytes.

The reports in the literature on this subject are unsatisfactory because of the incomplete and confusing data. About 22 reports of peptic ulcer situated exactly on the greater curvature apart from the pylorus were found in which the condition was described more or less accurately. Histologic examinations were mentioned in reference to 16 of these 22 cases. On the other hand, mention was made of 140 cases of the type concerned here in reports of 4,380 cases of peptic ulcer affecting all parts of the stomach. These 140 cases were mentioned only incidentally; the ulcers were not described and, so far as the reports indicate, were not studied histologically.

#### DISCUSSION

DALLAS B. PHEMISTER: I have no doubt that operations have been performed on a great number of patients with the mistaken diagnosis of carcinoma, as in these cases. In the second patient I suspected a perforating ulcer. There had been no infiltration of the entire stomach.

## Book Reviews

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**An Outline of Immunity.** By W. W. C. Topley, M.A., M.D., F.R.C.P., F.R.S., Professor of Bacteriology and Immunology, University of London. Price, \$6. Pp. 415, with illustrations. Baltimore: William Wood & Company, 1933.

The substance of a great part of the material in this book appears in Topley and Wilson's "Principles of Bacteriology and Immunity," which was reviewed in the ARCHIVES (9:1306 [June] 1930). "It has, however, been entirely rewritten. In some places it has been compressed, in others extensive additions have been made." The question of the need or desirability of this separate elaboration of the part on immunity in the earlier book is answered by the author's plea for more attention in the medical curriculum to the great advances in immunity, no matter what department may be entrusted with its teaching. On account of its practical importance, the fundamental principles should be taught in the preclinical years so that the student may be prepared for their applications to clinical medicine a little later. The prospective physician should have a basis of sound knowledge on which to develop his judgments on immunologic procedures in practice. Immunity is an applied science that enables the physician and the epidemiologist to intervene advantageously in the course of infectious diseases. It is from this point of view that the book has been written. In his introduction the author discusses the general relations of immunity to epidemiology and to clinical medicine, leading up to the consideration in the second chapter of the important and difficult matter of accurate measurement of immune conditions in the living. The need of sound statistical methods in immunologic work is made clear at the very beginning. A glance at each succeeding chapter, if only to note its title, will indicate the scope and general plan of the book. Chapters 3 and 4 deal with the mechanisms of bacterial infection and the mechanisms that hinder or prevent the access of bacteria to the tissues. The next six chapters are concerned with antigens and antibodies, their nature and their reactions; the antigenic structure of bacteria, knowledge of which has advanced rapidly in recent years; the mechanisms of antitoxic and of specific antibacterial immunity; the response of bacteria to the defenses of the host; the origin of the natural antibodies and their rôle in specific immunity, and the antibody-forming apparatus and its reactions. Chapter 12 reviews anaphylaxis, hypersensitiveness and allergy. As an example of the author's way of summarizing the evidence presented, the concluding paragraph of the summary at the end of this chapter is given in full:

"Acute anaphylaxis, natural hypersensitiveness, the allergic response and specific antibacterial immunity would appear to depend on the same fundamental mechanism—the union of an antigen with its specific antibody. The differences between them depend on the nature of the antigen concerned (living parasite, toxic substance or intrinsically inert material), the nature of the animal into whose tissues it penetrates, the rate and route of penetration, the distribution of the antibody in the animal's tissues and body fluids, the secondary effects of the primary cellular reactions, and a variety of other factors."

The three next chapters discuss nonspecific mechanisms in general immunity, local immunity and the influence of diet, fatigue, changes in temperature and humidity, chemical agents and other factors on immunity. The review of immunity in virus diseases (chapter 16) leads to the conclusion that there is no fundamental distinction between antiviral and antibacterial immunity. It is suggested that the intracellular confinement of viruses may play a rôle in the greater effectiveness of antiviral immunity. Then comes a chapter on herd infection and herd immunity, a complex process the understanding of which has been furthered by the investigations of the author himself. The concluding parts of the book are devoted to the

practical application of immunity in diagnosis (in the diagnosis of infection, of herd infection and of immunity), in prophylaxis (antitoxic, antibacterial and anti-virus immunization) and in treatment. The final chapter deals with the standardization of immunologic reagents.

This hurried account of the contents reveals the wide scope of the book. All phases of immunity, the scientific as well as the applied, are considered. The general plan is to set down the evidence fully and fairly and then to draw the conclusions that appear to be warranted. By means of different type forms the author indicates his ideas of the relative importance of the various parts of the text. Each chapter has a list of references, more copious than usual, so that those who are "wise enough to prefer their own conclusions to those offered by others may find access to the records." The style is clear and fluent. The author is the critical master of his subject in practically all parts. This does not mean that he may not be wrong in some of his conclusions, as, for instance, that there is only one specific streptococcus toxin. It is safe to say that no book written in English presents immunity in all its aspects so comprehensively and adequately as Topley's, but it goes over the head of the average medical student in the preclinical years. For the advanced student, the special worker and the teacher, however, there is no better book on immunity available at this time.

**Histology.** By S. Ramón y Cajal, M.D. (Madrid), F.R.S. (London), LL.D. (Clarke); Director, Royal Cajal Institute for Medical Research; Emeritus Professor of Pathology, University of Madrid Faculty of Medicine; Nobel Premiate in Medicine; Life Senator of Spain. Revised by J. F. Tello-Múñoz, M.D. (Madrid), Professor of Pathology, University of Madrid Faculty of Medicine. Authorized translation from the tenth Spanish edition by M. Fernández-Núñez, M.D. (Madrid), Professor of Pathology, Marquette University School of Medicine. Price, \$8. Pp. 738, with 535 illustrations. Baltimore: William Wood & Company, 1933.

From the translator's preface: "The present work is a translation of the elementary student's textbook of Prof. R. S. Ramón-Cajal, dean of Spanish histologists, for the purpose of presenting in English a compendium of the investigations of that author and his disciples." As was to be expected, the outstanding part of this book is the section devoted to the nerve tissue. This contains a summary of Cajal's views and many of his classic figures, and is further embellished by Tello-Múñoz' excellent illustrations of nerve endings. The other primary tissues are treated in some detail, but the microscopic anatomy of the organs is treated very briefly and in places the information is inadequate and out of date. For example, one would like to see more than ten lines devoted to the islands of Langerhans. A valuable feature of the book is the illustrations of the Golgi network in many unusual types of cells.

There are some points in the book which merit unfavorable criticism. According to the translator's preface, "the English version is an almost exact literal translation of the Spanish text, so that the nomenclature, phraseology and syntax will often appear unusual. This has been done with a view to rigid accuracy in translation, and the desire to enrich our histologic nomenclature by coining into English many of the vivid teaching locutions which made Professor Cajal's lecture room a Mecca for students." The reviewer cannot sympathize with the translator in his intention of enriching the English histologic nomenclature. The teacher and student of histology are sufficiently burdened with the present English terminology and the need for carrying the French and German equivalents. An unexpected disadvantage of this new terminology is the fact that the index is often of little aid in using the book, for it does not contain many of the common histologic terms (since these are not used in the text). The reviewer also objects to a translation so rigorous that Meves is spelled "Mevez" and Tomes "Tomez." The "almost exact literal translation" is incomprehensible in some places and inaccurate in others, as on page 30, the "Malpighian corpuscles of the skin." An accurate but freer translation would have made the text more easily understood.

There are many typographic errors, some of which are inexplicable, as, on page 30, the occurrence of "neutrophilic" and "eosinophylic" in the same line. Some of the figures taken from other authors are not credited to them, as figure 182 (von Ebner) and figure 189 (von Korff); in a few cases the figures have been credited in the text but not in a legend accompanying the illustration.

It is not fair to omit Altmann's name from the discussion of mitochondria and to credit the discovery to Benda. It is probable that most cytologists would disagree with the statement on page 16: "Concerning the function of the mitochondria most cytologists see in them the basic material entering into the formation of individual cells and serving as a basis of protoplasmic inheritance."

To the extent that the book calls attention to the works of the Spanish histologists, it is an addition to the English histologic literature. But the reviewer wonders whether many of the references to Cajal's investigations of forty years ago represent his views today—as, for instance, the idea expressed on page 609 regarding the hypophysis: "Cajal's investigations (1894) showed that the epithelium of the intermediary portion possesses the character of a sensory organ, since, besides containing certain cells, which can be considered homologous to the sensory epithelia of the organ of Corti, are also found arborizations of free nerves, terminating among the cells of the epithelial covering and proceeding from fibers arising in the nervous lobule."

Cajal's "Degeneration and Regeneration in the Nervous System" was translated into English in 1929. His "Histologie du système nerveux de l'homme et des vertébrés" is out of print. Perhaps a greater service would have been rendered English-speaking anatomists had this or other classic writings of Cajal been translated, rather than his elementary "Histology."

**Number 4 Canadian Hospital. The Letters of Professor J. J. Mackenzie from the Salonika Front. With a Memoir by his Wife, Kathleen Cuffe Mackenzie. Price, \$2.50. Pp. 247, with 13 illustrations. Toronto: The Macmillan Company of Canada, Ltd., 1933.**

Beginning with well written biographic notes, in which Mackenzie's descent from a distinguished Scottish line and his professional training under such men as Ramsey Wright, His, Braune, Karl Ludwig and Koch are outlined, there follow comments on his early career as professor of pathology at the University of Toronto. Many honors were conferred on him, including the presidency of the American Association of Pathologists and Bacteriologists (1914). His work, his inclination to sound problems of research and his interest in art, music and literature are interwoven in a philosophic vein, with an outlook on life and its meanings which will excite the sympathy of those who are closely bound by ties of deepest affection. The introduction merges into the description of the departure of Number 4 Canadian Hospital for its tour of duty abroad; then follow the letters from Salonika, which begin on Oct. 22, 1915, and continue practically daily to June 29, 1916. Mackenzie went back to London, where his wife had been waiting, and after a period of investigative work with Brodie returned to Toronto and his professional duties. The book closes with a brief summary of his life thereafter until his death in 1922 from streptococcic endocarditis.

The nature of the work of a scientist and the necessarily objective character of his scientific publications sometimes serve to conceal the traits of his personality even from those who have frequent professional contacts with him. The academic career is often pursued by men who are shy, and the seclusion in which much of their work is done does nothing to overcome this inherent reserve; and this was true of Mackenzie. With him, as with others of his kind, the quiet atmosphere of the writing desk served to unleash diffidence and to reveal the man himself. The letters are written in the simple and clear English of a person with a fine background, cultural refinement and a broad education. An undercurrent of love and devotion illuminates without dominating the more prosaic but deeply interesting account of the activities of the Hospital, the postmortem examinations, the bacterio-



logic problems, the journeys into the neighborhood, the critique of things political and military and the analytic comments on the extensive reading that could be done in those periods of quiet which intervene in the military hospital between the times of strenuous activity.

During the course of the World War the medical work of the armies was carried on in large part by those who, like Mackenzie, left pressing civilian duties and went overseas to labor in field and hospital. To all those this book will be a source of interest, enjoyment and appreciation. Most of the newer generation of doctors and nurses were too young in those stirring days to serve. To them the book will be instructive in its depiction of war without diagrams and maps of military actions, without figures as to divisions and regiments, without numbers of casualties and deaths, but war as it is seen in those tragic times when the sick and wounded are returned back of the lines for suffering and often death, for invalidism and for reconstruction. In this recountal the younger reader will find that valor in the face of dangers, seen and unseen, so conspicuous among the troops, was shared in no inconsiderable measure by the doctors and nurses and other members of the medical services of the armies. The book is almost devoid of technical terms and can be recommended also to the lay reader.

**Les troubles de l'élimination urinaire de l'eau. Etude physiopathologique et clinique.** By Jules Cottet, Ancien interne des hôpitaux de Paris. Price, 32 francs. Pp. 212. Paris: Masson & Cie, 1933.

Water is an important constituent of protoplasm and plays an active part in its metabolism. Cottet reports observations on tests of renal function, renal physiology, prerenal water metabolism and his clinical experiences with the water cure or provoked diuresis. He noted that the metabolism of water may be disturbed by abnormalities in absorption from the gastro-intestinal tract; by circulatory changes, especially cardiac insufficiency, and by excessive elimination through extrarenal pathways. These factors may be combined in infinite variety under clinical conditions. The normal kidney concentrates or dilutes the urine according to need, while the diseased kidney exhibits no functional rhythm but excretes urine of a fixed low specific gravity. Clinically, it is necessary to estimate in each case the renal secretory and diluting power, as well as the extrarenal factors affecting water metabolism. For this purpose the author studied the rhythm, quantity and specific gravity of the twenty-four hour urinary output of a patient who for five days previously had taken 5 Gm. of salt daily to establish an equilibrium between chlorides and water. The urine was collected and studied in three fractions: (1) a night specimen, from 9 p. m. to 7 a. m.; (2) a morning specimen, from 7 to 9 a. m., and (3) a day specimen from 9 a. m. to 9 p. m. The urine was measured; the color and specific gravity were recorded, and the amount of chlorides and urea determined. Also determinations of blood urea were made and compared with the urinary excretion of urea. Diuresis was provoked in the morning specimen by having the patient drink 600 cc. of water between 6:30 and 7:00 a. m. Normally a diuresis results which frequently exceeds the amount of water ingested. The amount of day urine was normally three times as great as that of night urine. With insufficiency of renal secretion there were absence or weakness of provoked diuresis, absence of orthostatic oliguria, an increase in night urine and fixation of density at a low level. With prerenal abnormalities in water metabolism there were marked orthostatic oliguria, excessive polyuria in the reclining position, equality in the amount of night and day urine and dissociation in the elimination of water and chlorides, that is, nocturnal polyuria, associated with diurnal polychloruria. The author describes and criticizes other functional tests, such as those of Violle, Bergouignan, Vollhard and Mosenthal. He discusses nycturia, orthostatic oliguria, urinary density, the rhythm of the excretion of chlorides and their clinical correlation with gastro-intestinal, hepatic, cardiovascular and renal disorders. Thus, he shows that cardiac insufficiency is characterized by marked oliguria, with uniformity of urinary volume and density. Urinary insufficiency may also be due to defective renal secretion, to retention of water and

chlorides or to oliguria of extrarenal causation. He describes mild types of oliguria and retention of urea due to a habitually inadequate intake of water. He cautions that in the therapeutic application of water in provoking diuresis, it is necessary to adapt the cure to the clinical condition of the patient, with particular regard to conditions of absorption, circulation and renal secretion. The book is written by a clinician; it is based on clinical studies and is chiefly for practitioners. As such it should be of value to all physicians interested in renal function and water metabolism.

**Physical Chemistry of Living Tissues and Life Processes as Studied by Artificial Imitation of Their Single Phases.** By R. Beutner, M.D., Ph.D., Professor of Pharmacology, School of Medicine, University of Louisville. Price, \$5. Pp. 337, with 79 illustrations. Baltimore: Williams & Wilkins Company, 1933.

This volume deserves a wider sale and reading than it will get—the former because the publisher has hazarded investment on a monograph, and the latter because it is concerned with cellular physiology, which is at once the most difficult and important phase of modern biologic study and the most “theoretical.” Beutner makes clear the problem in his opening sentences. The purpose of physiology is to explain the dynamics of a machine and the anatomic, chemical and physiologic analyses of the living machine are insufficient to this end. The physiologist or pathologist must therefore construct from the nonliving materials of the universe new machines (or models, as Beutner calls them) which “work” as does the living organism and by such “synthesis” build up systems that qualitatively and quantitatively are the analogs of living matter. Toward such an end Beutner makes three “attempts at approach,” discussing in the first, membranes and osmosis, in the second, crystallization and surface forces, and in the third, the electrical changes observed in living matter. The third is peculiarly that of the author (phase boundary potentials), though even in this he brings under discussion the related labors of other men, such as those of Lapique on “chronaxie,” of Ralph S. Lillie on wave transmission in iron wire and of Osterhout on electrical conduction in injured tissues. He is even more generous in discussing the findings and opinions of other authors in the remaining half of the volume. For this reason the modern biologic worker perhaps cannot be referred to a better volume than this for orientation on the mechanics of growth, the rôle of osmotic pressure, “membrane equilibrium,” the function of lipoids, colloid swelling (very weak), intracellular oxidation, cellular “permeability,” emulsions, protoplasmic structure, “micelles,” solid and liquid crystals, surface tension and adsorption. Beutner thus demonstrates how in the nonliving world the most various types of systems are known which, without recourse to meaningless “vital” explanations, give a clew to what may be happening in the living. But he attempts little critique of mutually exclusive findings or opinions and makes no effort to coerce the reader into any one pathway of biologic thought. The synthesis of what living matter may be after all is therefore largely left to the reader himself, and he is repeatedly warned against premature conclusion by Beutner’s constantly reiterated, “But of this matter we know nothing.”

**L'infection chez les insectes; immunité et symbiose.** By A. Paillot, Docteur ès-sciences, Lauréat de l'Institut; Directeur de la Station de Zoologie Agricole du Sud-Est. Cloth. Price, 100 francs. Pp. 535, with 279 figures. Lyon: Librairie Médicale et Scientifique, 1933.

The scope and general nature of this interesting book can be illustrated by briefly indicating its subdivisions. There are seven parts. The first four deal with infections of insects by protozoa, fungi, ultraviruses and bacteria, respectively. The fifth part is devoted to the consideration of natural and acquired antibacterial immunity in invertebrates. In the sixth part the author discusses symbiosis in the plant louse (*Pucceron*). In the seventh part certain economic

considerations of infections in insects and the rôle of insects and other arthropods in the transmission of infections are discussed. No attempt will be made to review the contents in detail. The presentation is clear, direct, orderly. The book is well printed and well illustrated. Undoubtedly, the study of the diseases of insects due to ultraviruses is bringing to light new facts that may contribute to a better understanding of these diseases in general. Antibacterial immunity in invertebrates appears to be associated in a large degree with humoral phenomena, and phagocytosis seems to play only an insignificant rôle. In certain insects symbiosis may be regarded as natural antibacterial immunity. In view of the interest in the rôle of insects in transmitting infectious diseases to man, animals and plants, Paillot's book will be heartily welcomed. It is an important and stimulating contribution to the comparative pathology of infectious diseases.

**The Anatomy of the Rhesus Monkey (*Macaca Mulatta*).** By T. H. Bast and others. Edited by Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington, and William L. Straus, Jr., Department of Anatomy, Johns Hopkins University, Baltimore. Cloth. Price, \$6. Pp. 383, with 128 illustrations. Baltimore: Williams & Wilkins Company, 1933.

This book has been produced by the joint efforts of interested scientists to meet the growing need for a handbook on the anatomy of the rhesus monkey. It is the first comprehensive work on the anatomy of an Old World monkey. Each of the eighteen chapters has been written by an especially qualified author or authors actively engaged in research closely related to the subject in question. There is an appendix on the housing and care of monkeys. The illustrations, almost all of which are drawings by Benjamin Kopel, are excellent. At the end of each chapter is a selected bibliography. The terminology of human anatomy (Basle anatomic nomenclature) is used. There is no apparent reason for disagreeing with the editors in their statement that "the book attains the immediate object of affording a useful description of the anatomy of the rhesus monkey for research workers as well as for such classes in comparative anatomy as contemplate using this animal as the object of dissection."

**Atlas of Pathological Anatomy.** Volume I. Compiled by E. K. Martin, M.S., F.R.C.S. Issued under the direction of the editorial committee of the British Journal of Surgery. Price, \$15. Pp. 489. Baltimore: William Wood & Company, 1933.

This atlas contains the drawings of pathologic specimens published in the supplement to the *British Journal of Surgery* during the five year period ending with 1930. The object of the compilation is to foster a greater interest by surgeons in morbid anatomy, especially as related to clinical surgery. The supply of suitable material for this purpose is unlimited. The specimens illustrated in the volume are from the great Hunterian Museum of the Royal College of Surgeons and, to a less extent, from museums of medical schools. The volume covers tumors of bone, diseases of the stomach, breast, kidneys and gallbladder and bile ducts and inflammations of bone. Not rare conditions, but typical examples of well known diseases, are represented. The text consists of brief descriptions of the gross and microscopic material, followed by a brief statement of the clinical features. There is no statement as to the preparation of the microscopic sections. The pictorial part presents a large number of good colored illustrations of gross specimens and also black and white drawings of gross specimens and typical microscopic fields. There are reproductions of a few roentgenograms. Generally speaking, all the reproductions are excellent. The microscopic drawings in some cases are perhaps too diagrammatic. It is planned to continue the publication until it will include practically all subjects that can be illustrated with museum specimens. Undoubtedly, the atlas will help to interest the surgeon in a closer study of the structural characteristics of the conditions that come under his hand and eye.

**The Biology of Bacteria: an Introduction to General Microbiology.** By Arthur T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Price, \$3.60. Pp. 472, with 112 figures. Boston: D. C. Heath & Company, 1934.

The preface states that "this is a textbook to be used by general or nontechnical students who will take but one course in microbiology; or in introductory courses for other students who will be taught particular applications of the science in advanced courses." The book, then, stresses fundamental knowledge of the subject rather than application. As indicated by the title, it deals for the most part with bacteria, but other microbes also receive proper consideration. The first sixteen chapters deal with the history of bacteriology; the microscope; a survey of microbic life (protozoa, algae, fungi, bacteria and ultramicrobes); the finer structure, growth and reproduction, heredity and variation, metabolism, cultivation and death of bacteria, and infection and immunity, including bacterial diseases of plants. Then comes a chapter on the classification of bacteria, which is followed by chapters on Nitrobacteriaceae, Coccaceae, Spirillaceae, Bacteriaceae, Bacillaceae, Mycobacteriaceae and Actinomycetaceae. As indicated, the classification of the American Society of Bacteriologists (Bergey's Manual) is followed. The final chapters are devoted to molds, yeasts, pathogenic protozoa, Spirochaetales, Rickettsiae and virus diseases. At the end of each chapter are brief suggestions for further reading. The illustrations, original and borrowed, are good. The style is clear and pleasant. The book is recommendable without reservation as a sound and useful textbook in its field.

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#### CORRECTION

An error occurred in the last sentence of the abstract of the article by C. V. Harrison entitled "Arterial Disease Produced by Cholesterol and Vitamin D" in the January issue. This sentence should read: "It is concluded that the movements of the vessel determine the localization of both vitamin D and cholesterol lesions and probably also human arterial disease."

## Books Received

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PRÉCIS DE MICROSCOPIE. M. Langeron, Chef de laboratoire à la Faculté de Médecine de Paris; Chef des travaux de parasitologie à l'Institut de médecine coloniale. Cinquième édition, revue et augmentée. Paper, 86 francs; cloth, 100 francs. Pp. 1205, with 355 figures. Paris: Masson et Cie, 1934.

THE BIOLOGY OF BACTERIA: AN INTRODUCTION TO GENERAL MICROBIOLOGY. Arthur T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Price, \$3.60. Pp. 472, with 112 figures. Boston: D. C. Heath & Co., 1934.

THE THYROID GLAND: ITS CHEMISTRY AND PHYSIOLOGY. Charles Robert Harington, M.A., Ph.D., F.R.S., Professor of Pathological Chemistry in the University of London. Pp. 222, with 28 figures. London: Oxford University Press, 1933.

ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH FOR THE YEAR 1932. A. Neave Kingsbury, Director, Institute for Medical Research, F.M.S., Kuala Lumpur. Pp. 108. Kuala Lumpur: Federated Malay States Government Press, 1933.

PUBLICATIONS OF THE DEPARTMENT OF PATHOLOGICAL ANATOMY AND HISTOLOGY OF THE HUNGARIAN ROYAL FRANCIS JOSEPH UNIVERSITY, SZEGED, HUNGARY. Volume 3. Edited by Josef Baló. Szeged, 1933.

LES TUMEURS BÉNIGNES DU LARYNX: ÉTUDE ANATOMIQUE ET CLINIQUE SUIVIE D'UNE BIOGRAPHIE DE L'AUTEUR. Professeur Ricardo Botey. No. 25, Monographies oto-rhino-laryngologiques internationales. Publiées par M. Vernet, L. Ledoux, G. Portmann, H. Aloin et M. Sourdille. In memoriam. Paper. Price, 25 francs. Pp. 79, with 26 illustrations. Paris: Les Presses Universitaires de France, 1932.

L'ETHMOÏDITE. Patrick Watson-Williams et Eric Watson-Williams. No. 22 Monographies oto-rhino-laryngologiques internationales. Publiées par M. Vernet, L. Ledoux, G. Portmann, H. Aloin et M. Sourdille. Analyse en anglais. Paper. Price, 35 francs. Pp. 144, with illustrations. Paris: Les Presses Universitaires de France, 1932.

A STUDY OF GROWTH AND DEVELOPMENT: OBSERVATIONS IN SUCCESSIVE YEARS ON THE SAME CHILDREN. R. M. Fleming, with a Statistical Analysis by W. J. Martin. Medical Research Council, Special Report Series, No. 190. Price, 1s. 6d. Pp. 85. London: His Majesty's Stationery Office, 1933.

THE LYOPHILIC COLLOIDS (THEIR THEORY AND PRACTICE). Martin H. Fischer, Professor of Physiology in the University of Cincinnati, and Marian O. Hooker, Research Associate in Physiology in the University of Cincinnati. Price, \$4.50. Pp. 250, with 84 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

NEUROLOGY. Roy R. Grinker, M.D., Associate Professor of Neurology, University of Chicago. Price, \$8.50. Pp. 974, with 401 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

DIET AND THE TEETH. AN EXPERIMENTAL STUDY: PART III. THE EFFECT OF DIET ON DENTAL STRUCTURE AND DISEASE IN MAN. May Mellanby. Medical Research Council, Special Report Series, No. 191. Price, 5s. Pp. 180, with 46 plates. London: His Majesty's Stationery Office, 1934.

A DIABETIC MANUAL FOR THE MUTUAL USE OF DOCTOR AND PATIENT. Elliott P. Joslin, M.D., Clinical Professor of Medicine, Harvard Medical School: Medical Director, George F. Baker Clinic for Chronic Disease at the New England Deaconess Hospital; Consulting Physician, Boston City Hospital, Boston. Edition 5, thoroughly revised. Price, \$2. Pp. 224, illustrated. Philadelphia: Lea & Febiger, 1934.

## EXPERIMENTAL ATHEROSCLEROSIS IN THE RABBIT COMPARED WITH HUMAN (CORONARY) ATHEROSCLEROSIS

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The literature on arteriosclerosis has been so recently and thoroughly reviewed in the book on this subject issued by the Josiah Macy, Jr., Foundation<sup>1</sup> that a detailed discussion is unnecessary. The *lipoid* relations of the common form of the disease have been investigated by chemical tests which revealed the high content of lipoids in atheromatous aortae. The Aschoff-Virchow theory separated atherosclerosis from the other forms of arteriosclerosis. The experimental production of atherosclerosis in rabbits by feeding them cholesterol, reviewed by Anitschkow,<sup>1</sup> was largely the work of the Russian school. Wolkoff<sup>2</sup> studied the evolution of changes in human coronary arteries with age, the distribution of lipoids in these arteries and their branches, and the production of experimental coronary atherosclerosis in the rabbit.

Practically all of the chemical work, most of the experimental research and much of the morphologic studies have dealt with the lesions of the aorta. From the standpoint of its effect on human life atherosclerosis of the aorta is of relatively minor importance. Moreover, the aorta is of highly complex structure histologically and is extremely rich in elastica. Atherosclerosis of the coronary arteries is of primary importance from the point of view of human disease. The coronary arteries are of relatively simple structure histologically; they are muscular arteries, poor in elastica. The simplicity of structure of these vessels permits of more exact appraisalment of the origin, progress and character of the lesions of atherosclerosis than is possible in the aorta.

The coronary arteries differ from other muscular arteries in developing a layer of unstriated muscle and cellular subendothelial fibrous tissue. This is without a circulation of its own, depending for nutrition on imbibition

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Medical Association.

1. Anitschkow, in Cowdry, Edmund V.: *Arteriosclerosis: A Review of the Problem*, New York, The Macmillan Company, 1933.

2. Wolkoff, C.: (a) *Virchows Arch. f. path. Anat.* **241**:42, 1923; (b) *Beitr. z. path. Anat. u. z. allg. Path.* **82**:555, 1929; (c) *ibid.* **85**:386, 1930.

through the endothelium. That the buffer layer in the coronary arteries is a response to the unusual stresses which these vessels are called on to bear seems reasonable. It is now generally agreed that the main coronary vessels and their branches, which lie in the subepicardial fat, are filled with blood during systole. Per contra, the finer branches in the myocardium are compressed and may be closed by the contracting muscle during systole. At the end of systole the aortic cusps are closed and the highest point of aortic pressure is momentarily produced. It is this peak pressure which is believed to cause the rupture of aortic aneurysms. With the diastolic relaxation of the heart muscle the intramural branches of the coronary arteries are freed from compression and are filled with blood, the maximal flow occurring during this period. Throughout a part of the cardiac cycle the main coronary arteries and their epicardial branches are meeting high pressure from the blood-filled aorta and resistance from their compressed intramural branches.

It is recognized that the proximal inch of the left coronary artery and its descending branch is the favorite region of localization of coronary atherosclerosis in its most extreme form. This is the standard site of coronary thrombosis. A probable factor of importance in this regard is the character of the curve which this part of the vessel takes in its descending course. Coming off at right angles to the aortic wall, this portion of the vessel describes a curve which brings it rather abruptly to a course parallel to that of the aortic wall. It is believed that the localization of atherosclerosis and of syphilitic aortitis with resulting aneurysms in the aortic arch is related to the curve which the aorta takes and to the high and varied pressures produced in this part of the vessel. The curve of the left coronary artery in its proximal portion is quite sharp and, perhaps, comparable to that of the ascending portion of the aortic arch. Moreover, a large branch of the left coronary artery, the circumflex, arises near the orifice at right angles to the main vessel. The right coronary artery changes direction only slightly from its origin, is usually a relatively straight vessel for a large part of its course, and gives off no large branches in its proximal portion.

In the following paper three series of observations are recorded:

- I. Human coronary sclerosis.
- II. Experimental atherosclerosis in the rabbit
- III. A comparison of human and experimental rabbit atherosclerosis in the coronary arteries

#### I. HUMAN CORONARY SCLEROSIS

The study of a large series of coronary arteries, lesions of which had led to a fatal termination, demonstrated that the histologic changes

which the vessels had undergone caused them to fall into two groups. This histologic division corresponded closely to a difference in the ages of the subjects represented in the two groups. The first group, in which the dominant change in the coronary arteries consisted of fibrosis leading to narrowing of the lumen, represented persons from 25 to 55 years of age. The second group in which the dominant change consisted of collections of lipid cells with a tendency to atheromatous necrosis, giving rise to so-called atheromatous "abscesses," represented persons from the age of 47 years up to extreme old age.

There were two exceptions to this rule in the series, occurring in young men, but a reason for these exceptions is made clear in the case records (cases 20 and 21).

It is indicated that the lesions in the first group represent a reaction of youth. The lesions in the second group depend apparently on a lack of this reaction at older ages.

The following cases were selected only because of the necessary limitations of illustration, save that in the first group the cases of the youngest patients have been reported.

#### GROUP 1 (FIG. 1)

CASE 1.—A man, aged 25, an athlete, who had had no previous illness save one attack of indigestion two weeks before death, was playing baseball as usual on Sunday, the day of his death. He made a hit, ran to second base and collapsed. The heart weighed 405 Gm. The conditions found were: *high grade sclerosis of the left coronary artery, descending branch* (fig. 1 A), *with thrombosis at a higher level*; fibrosis with occlusion of the circumflex branch; moderate sclerosis of the right coronary artery, and myocardial atrophy with repair (chronic myocarditis) near the apex. The aorta was thin, smooth and elastic save for one raised scar, 2.5 cm. in diameter, 3 cm. above the bifurcation; the aorta showed a rather diffuse yellow opacity, notably about the orifices of vessels, and several small gray regions with little projection.

CASE 2.—A man, 26 years of age, a chauffeur, had had an occasional attack of acute indigestion. He was removing a wheel from an automobile when he collapsed and within a few minutes, when a doctor could be found, was pronounced dead. The heart weighed 415 Gm. and presented a *high grade sclerosis with thrombosis of the descending branch of the left coronary artery* (fig. 1 B). There was also marked sclerosis of the circumflex branch and the right coronary artery. The aorta was thin, smooth and elastic, with delicate yellow streaks along the lines of the intercostal orifices—not raised.

CASE 3.—A man, 28 years old, a clerk, felt a sudden onset of precordial pain and anxiety. He sat bent forward with hands on chest at home for four hours. He was admitted to a hospital, where he sat in bed in a similar position for one hour and twenty minutes before death. The heart weighed 340 Gm. and showed *fibrous sclerosis and thrombosis of the descending branch of the left coronary artery for 1 cm., beginning 1.5 cm. from the orifice* (fig. 1 C). There was gross occlusion of the circumflex branch 3 cm. from the orifice. Opaque yellow patches were scattered throughout all the coronary vessels, but the lumens elsewhere were free. The aorta was thin, smooth and elastic.



CASE 4.—A man, aged 31, a stationary engineer, did not feel well, and drank tea and chicken soup. He lay down and "had a fit," dying suddenly about one hour and fifty minutes after he began to feel badly. There had been no previous



Fig. 1.—Group 1. Fibrosis, the reaction of youth. Thromboses in cases 1 to 8 are shown in A to H inclusive. Coronary insufficiency in case 9 is shown in I.

symptoms. The descending branch of the left coronary artery showed eccentric thickening with thrombosis over a region 0.7 cm. long, beginning 1.3 cm from the orifice (fig. 1D). The aorta was smooth and elastic, with two thick circular fibrous plaques in the arch.

CASE 5.—A woman, 34 years of age, married, had nephritis of pregnancy one year before death. Several years prior to death she had had an operation for gallstones. Four days before death she had pain in the left side of the chest extending to both arms and upward to the neck. No dyspnea accompanied this. She went to a physician's office. The pain was relieved by morphine. The pulse was of good quality and regular during the attack. She suffered a second attack of pain two days before death, also relieved by morphine. The pain recurred early in the morning. While talking with her husband she suddenly collapsed and died. The heart weighed 400 Gm. and showed acute fibrinous pericarditis. There was an infarction of the wall of the left ventricle from the apex upward, including the left portion of the septum, to within 2 cm. of the aortic cusps. *The descending branch of the left coronary artery showed eccentric thickening, beginning 1.5 cm. from the orifice. The lumen was closed by a thrombus 2 cm. from the orifice (fig. 1 E).* There was generalized patchy thickening of the walls of both coronary arteries, most marked in the left coronary artery. The aorta was thin, smooth and elastic save for a scar 5 cm. by 1 cm. in the abdominal portion of the vessel and a few small plaques of lime salts above the bifurcation. There were regions of yellow opacity without projection about the orifices of the branches.

CASE 6.—A man, 37 years of age, a laborer, who had had no symptoms, but who had been drinking alcoholic liquor, went to bed at 11 p. m. At 2:45 a. m. he snored so violently that his wife became alarmed and called a doctor who on arrival pronounced the man dead. The heart weighed 430 Gm. *In the left coronary artery together with the descending and circumflex branches there was eccentric thickening, with thrombosis of the descending branch near the origin (fig. 1 F).* The right coronary artery showed some thickening of the wall, but the lumen was large and clear. The aorta showed patches of yellow opacity, without thickening about the ring, and pinhead-sized yellow opaque regions scattered in the arch, about the orifices of the intercostal branches and almost continuous in the abdominal portion of the vessel. Apart from these deposits, which did not project, the aorta was thin, smooth and elastic.

CASE 7.—A man, aged 38, an attendant in an automobile parking space, with no previous illnesses, went into a restaurant, complaining of indigestion, and asked for sodium bicarbonate in water, which he drank. He walked a short distance to an express office, sat down in a chair and then collapsed on the floor—all within ten minutes. The heart weighed 390 Gm. The coronary orifices were small, in scarred areas in the aortic ring. *The descending branch of the left coronary artery was narrowed almost to occlusion with thrombosis for 0.8 cm., beginning 1.5 cm. from the coronary orifice (fig. 1 G).* Patches of irregular thickening were scattered through the coronary vessels. A series of raised gray scars and yellow opaque foci were present in the thoracic and upper abdominal regions of the aorta. A ruptured atheromatous focus, 2.5 cm. by 2 cm., covered with translucent gray-red clot overlying red clot, was found in the midabdominal part of the vessel.

CASE 8.—A man, 38 years old, an automobile mechanic, lived at the top of a hill 100 ft. (30 meters) high, with a steeply graded approach. He came up the hill rapidly and said that his heart bothered him. He later got out of bed and sat at a window two hours. The next day he saw a physician, who found a tachycardia, which was relieved by morphine. Two days later he was seized with pain in the chest and took a Seidlitz powder. He suddenly fell to the floor and when a doctor arrived, within a few minutes, he was pronounced dead. The heart weighed 320 Gm. *The descending branch of the left coronary artery was found*

*almost completely occluded for 2 cm., beginning 2 cm. from the coronary orifice, with a sharply localized clot 2.5 cm. from the orifice (fig. 1 H).* The proximal portion of the circumflex branch was similarly narrowed. The condition of the right coronary artery was not recorded. The aorta showed opaque yellow patches in the descending arch, with streaks along the intercostal orifices. There were several gray scars in the abdominal portion of the vessel.

CASE 9.—This was an instance of coronary insufficiency. A man, aged 41, a mechanic, had had neuritis for one year. Apart from this he had not had any reason to consult a doctor for many years. There had been no indigestion. He started to walk to work with a fellow workman on a cold morning. He collapsed on the street and died suddenly. The heart weighed 370 Gm. There was eccentric yellow thickening in regions of the wall of the left coronary artery, but the lumen was of moderate size and clear. *In the right coronary artery there was almost complete obliteration of the lumen for 2 cm., beginning 2 cm. from the orifice. There was no thrombosis (fig. 1 I).* Beyond this point the walls were slightly thickened and showed some calcification. The arch of the aorta was beset with many pinhead-sized yellow foci. In the thoracic region occurred yellow streaks and pale gray larger patches over deep yellow deposits. The surface over the pale gray regions was edematous, and section disclosed mucoid swelling of the tissues. In the abdominal portion of the vessel similar patches of gray scarring and several larger soft atheromatous foci were present.

#### SUMMARY OF OBSERVATIONS IN GROUP 1

*Nutrition of the New Tissue.*—The nutrition of the new fibrous tissue in this group depended in its early stages on imbibition from the blood circulating in the lumen. Ultimately, as the fibrous layer increased, this became inadequate and necrosis resulted. In each of the vessels shown in figure 1 there was a zone of necrosis, which tended to be crescentic and which affected the layer farthest from the lumen, i. e., next to the media. In coronary artery *E* (fig. 1) there was massive necrosis including most of the new tissue. In the coronary arteries exhibiting the standard crescentic necrosis small groups of cells directly next to the media sometimes survived, particularly if vasa vasorum were present.

More interesting, however, was the formation in the more central layers, near the lumen, of a capillary circulation. This capillary layer seemed to be cut off by the zone of necrosis from any connection with the outer wall and a circulation through possible vasa vasorum. Search of series of sections revealed a direct connection of this capillary layer with the coronary lumen. This is best illustrated in the coronary artery of a painter, 55 years of age, who died suddenly without previous symptoms (case 10). Here the central capillary zone was large (fig. 2). Such a capillary circulation was found in coronary arteries *B*, *C*, *D* and *G* (fig. 1). In coronary artery *A* no vessels were found in the new intimal tissue. In coronary artery *G* vasa vasorum supplied the outer layer as well. In coronary arteries *F*, *H* and *I* what circulation was present came from vasa vasorum. In coronary artery *E* no vasa

vasorum were found, but groups of cells survived along the medial junction in a relation suggesting their possible influence.

*Elastica.*—As was to be expected, the internal elastic lamina in the advanced processes in these terminal conditions showed fragmentation and flattening. It even disappeared in regions where the fibrous layer

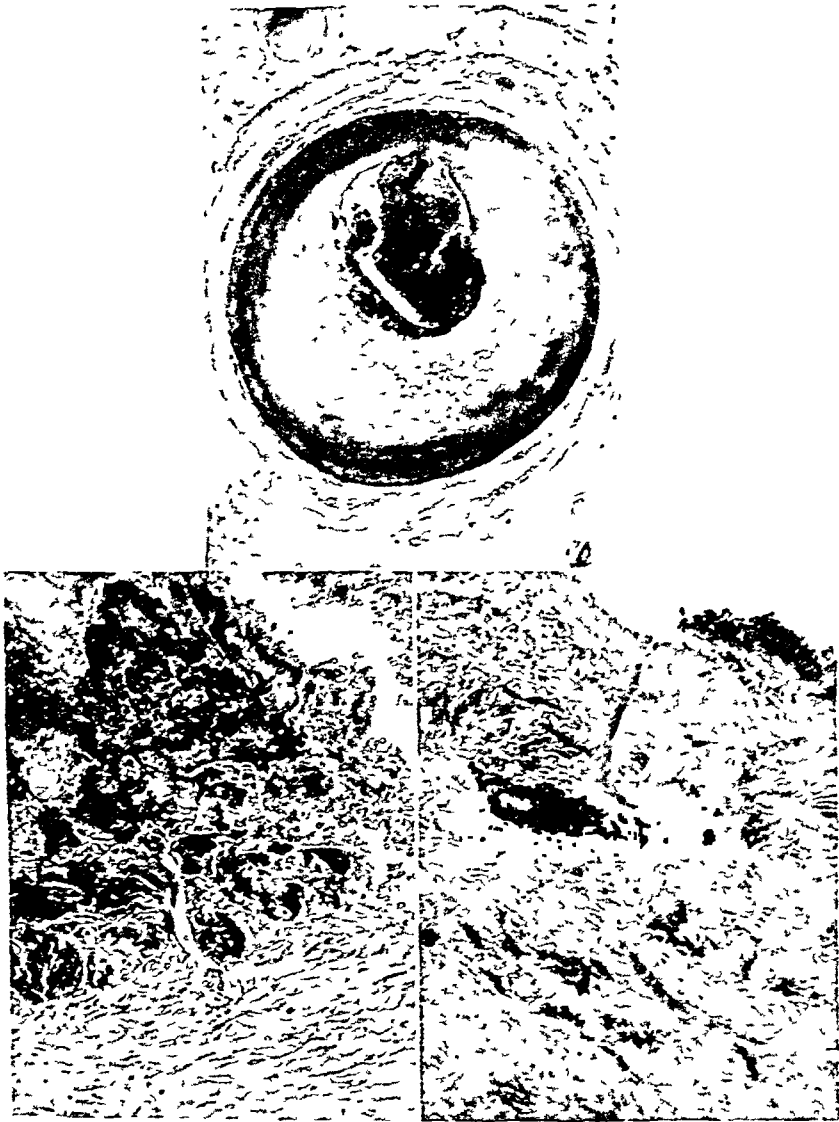
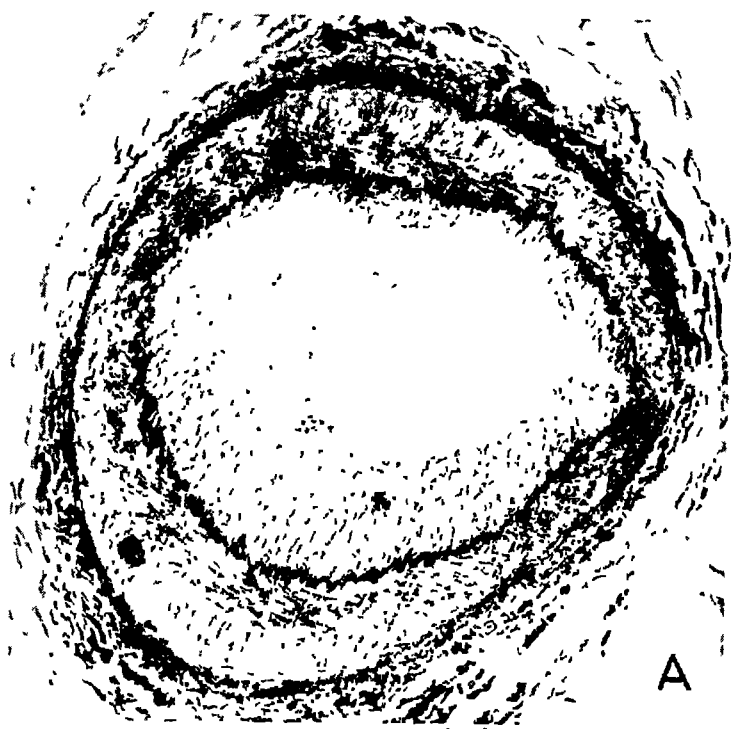


Fig 2—Central capillary circulation connecting with lumen of coronary artery in case 10, with two capillaries opening on surface.

had crossed its site and invaded the media. Only occasionally was there evidence of reduplication.

Sections from smaller branches of the coronary arteries, in which the process was seen in earlier stages, disclosed in the earliest processes an almost intact elastic lamina (fig. 3 *A*). Even in advanced processes



A



B

Fig. 3—*A*, branch of human coronary artery;  $\times 90$ ; early fibrosis with lipid cells; elastica almost intact. *B*, left descending branch of human coronary artery;  $\times 50$ ; advanced fibrosis, elastica in normal locus, somewhat fragmented and broken; media intact.

the elastic lamina was sometimes found in its normal locus and, though somewhat flattened and fragmented, gave evidence of its resiliency by its wavy outline (fig. 3 *B*). The principal changes in the elastica occurred where fibrous tissue from the intima invaded its site. While conditions of stress favor the localization of lesions in the arteries, definite morphologic evidence of injury to the elastica is not constant.

*Media.*—The media in the left coronary artery in case 2 (fig. 1 *B*) showed general hypertrophy. Hypertrophy in part was shown in others, as in that in case 1 (fig. 1 *A*). There was invasion of the media by connective tissue in the lower segment of this artery, and varying degrees of local invasion, thinning or hypertrophy in all of the others of the group. As was true of the elastica, examination of sections of early lesions disclosed no evidence of medial injury or reaction. The lesions of the media appear to be secondary to the intimal process.

*Cellular Inflammatory Reaction.*—Varying degrees of lymphoid cell infiltration of the intima were seen in this group. In the left coronary artery in case 1 (fig. 1 *A*) a rare lymphoid cell occurred in the more central portion of the thickened intima, and a rare small group near the media. In the other arteries in this group a more considerable degree of infiltration appeared, particularly near the regions of necrosis, and in some cases near the thrombosed lumen.

There was no lymphoid cell infiltration of the media in the left coronary arteries in cases 1, 2, 3, 4, 5 and 8 (fig. 1 *A, B, C, D, E* and *H*). There were focal collections of cells in the artery in case 6 (fig. 1 *F*) and diffuse infiltration in that in case 7 (fig. 1 *G*).

Perivascular collections of lymphoid cells occurred in the adventitia of all of the coronary arteries in the group save those from cases 1 and 4 (fig. 1 *A* and *D*).

Studies of early processes in branches of the coronary arteries, where the lesions were still proliferative and without necrosis, revealed no lymphoid cell invasion or other evidences of inflammatory reaction.

The relation of the lymphoid cell infiltration to the advanced lesions, in which necrosis had occurred, makes it probable that the inflammatory reactions arise in this disease as a response to the presence of necrotic tissue products. The inflammatory reaction is therefore secondary and usually late. This is consistent with the finding of MacCallum<sup>1</sup> that infections probably do not play a great part in the pathogenesis of arteriosclerosis.

*Calcification.*—Tangible deposits of lime salts did not occur in the coronary arteries of the first group. A minor degree of calcification was found in the arteries from some of the older cases of group 2. Calcification is in general a much later phenomenon in the coronary arteries than in the aorta. From the standpoint of etiology it is of little

significance. Jenner's famous letter has influenced clinicians to look on calcification as of particular importance in coronary disease. The tissue deposit of lime salts is always of monumental character, marking the site formerly occupied by living tissue. In some cases of coronary disease it may serve, as an end-result, to prevent coronary spasm, and thus, perhaps, delay the fatal termination.

*Lipoid Cell Infiltration.*—Lipoid cell groups, usually small, were found in all the coronary arteries in this group, and definite atheromatous regions of liquefaction were present in the coronary arteries from cases 6 to 9 (fig. 1 *F, G, H* and *I*). The process was dominantly fibrous, however, even in these vessels, particularly about the lumens.



Fig. 4.—Subendothelial V-shaped region of necrosis from case 1; stained with Weigert's stain for fibrin.

The intimate relation of lipoid cells to the new growth of fibrous tissue was manifest in all of these vessels. It was best illustrated, however, in early lesions in the smaller branches of the coronary arteries. Discussion of this relation is deferred to the second section of this paper where comparative illustrations make the process more clear.

*Cause of Thrombosis.*—In the left coronary arteries in cases 1, 2, 4, 5, 6 and 8 (fig. 1 *A, B, D, E, F* and *H*) there occurred regions of fibrinous or fibrinoid<sup>3</sup> necrosis arising in the subendothelial tissues and extending to the intima (fig. 4). The material saved in cases 3 and 7

3. The material reacts in some cases completely and in others incompletely to Weigert's stain for fibrin.

was minimal; it is possible that further sections would have disclosed this lesion. Its occurrence in six of the eight arteries illustrated, a percentage which corresponds to that found at older ages, suggests that this is the lesion which leads to thrombosis when the necrosis reaches the endothelial layer. This reasoning is based on the belief that endothelial necrosis leads to thrombosis. The subendothelial and the endothelial processes may have been produced simultaneously. It is difficult to account for the subendothelial lesion as a sequence of an endothelial necrosis.

GROUP 2 (FIG. 5)

CASE 11.—A retired business man, 61 years of age, with no record of illness, collapsed on the street. He was brought to the hospital by the police and there was pronounced dead. The heart weighed 425 Gm. The coronary arteries showed regions of narrowing almost to occlusion, notably of the descending branch of the left coronary artery and particularly just below the origin of the circumflex branch. The circumflex branch showed thickening with calcification, as did the right coronary artery. The size of the lumen in some regions was, however, almost normal. The heart showed atrophy with fibrosis over the left wall of the left ventricle extending onto the septum, with atrophy of the left papillary muscle. There was high grade sclerosis, with scarring and plaques of lime salts, throughout the aorta. Figure 5 *A* shows an *uninterrupted atheromatous "abscess" in the proximal portion of the descending branch distorting the lumen, which lay to the left and above. The vessel was obliterated by fibrosis below.*

CASE 12.—A man, aged 50, an engineer in an ice plant, with no previous complaints, had a pain in the chest and was taken by his son in an automobile to a doctor's office. He collapsed in the automobile and was pronounced dead. The heart weighed 380 Gm. There was narrowing of the descending branch of the left coronary artery almost to occlusion 1.5 cm. below the orifice. Below this the lumen was the size of a small pin; it then widened to a moderate size. In the circumflex branch and the right coronary artery occurred patches of yellow thickening, but the lumen of the right coronary artery was large. The aorta in general was smooth, with a large scar at the junction of the ductus arteriosus. Yellow opaque regions without projection were present in the arch and in the thoracic regions of the vessel, and slightly raised opaque yellow regions in the abdominal portion of the vessel, with three scars, two of them large, extending into the iliac vessels. Figure 5 *B* shows a *large, almost empty, atheromatous "abscess" to the right and below. The lumen to the left and above contains a thrombus including lipid cells.*

CASE 13.—A man, 60 years of age, an appliance mechanic employed by a gas company, had had pain in the chest for two weeks. He went to work at 9 a. m., returned home at 11 a. m., and had a typical attack of angina. He was found dead at 5.15 p. m. The heart weighed 656 Gm. In the descending branch of the left coronary artery was a clot 2 cm. from the orifice in a region of narrowing. In the lower two thirds of its course the vessel showed almost complete obliteration. The circumflex branch and the right coronary artery showed minor sclerosis. The myocardium was thinned, with fibrosis over the left wall of the left ventricle and the neighboring septum. In the aorta there was a relatively minor degree of sclerosis, with rather abundant yellow opaque patches. Figure 5 *C* shows the *point of rupture of an atheromatous "abscess," to the right and below,*



into the lumen, to the left and above. Thrombosis and atheromatous contents were found below this level.

CASE 14.—A man, aged 51, an inspector of water meters, collapsed in a house after coming upstairs from the cellar, where he had inspected the meter. There

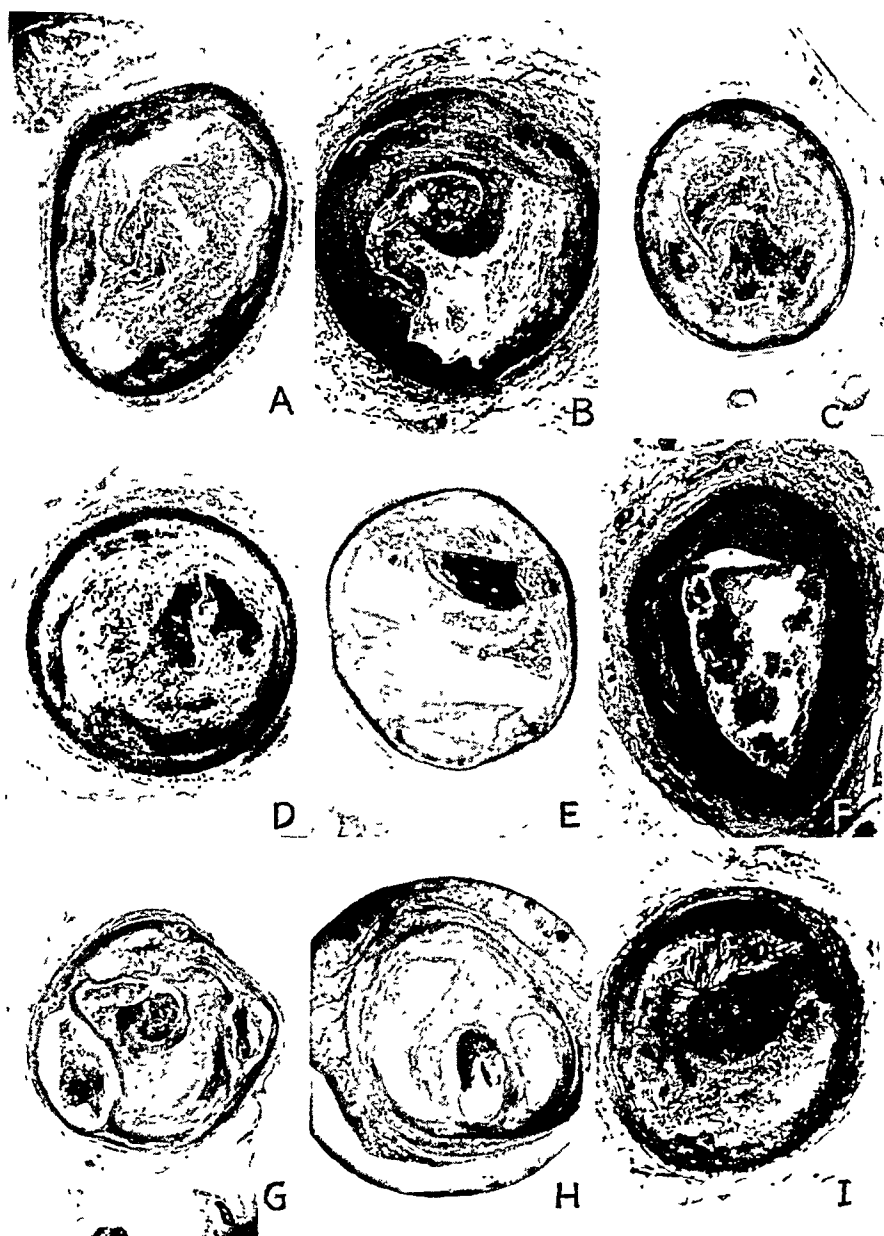


Fig. 5.—Group 2. Atheromatosis—lack of reaction in age. Rupture of or into atheromatous pockets, with thrombosis, in all cases save case 11 (A) and case 19 (I). In the latter cases death was due to coronary insufficiency.

was no history of illness. He was pronounced dead on arrival at the hospital. The heart weighed 400 Gm. There was scarring of the aortic ring with narrowing about the coronary orifices, notably the right. There was narrowing of the lumen of the descending branch of the left coronary artery, with almost

complete occlusion 2 cm. from the orifice. In the circumflex branch there was eccentric thickening of the wall. The right coronary artery, a vessel of normal caliber beyond the narrowed orifice, exhibited minor thickenings of the wall with a clear lumen. Moderate sclerosis of the aorta was present about the orifices of branches. Figure 5 *D* shows a large atheromatous "abscess" to the right and below rupturing through necrotic regions, shown by deep staining fibrin, into the narrow lumen above to the right. A platelet clot is seen in the lumen.

CASE 15.—A man, 58 years old, a shoe manufacturer, was attending a convention. He had had slight indigestion at times. He collapsed in a hotel and was pronounced dead by a physician almost immediately. The heart weighed 320 Gm. There was narrowing of the lumen of the descending branch of the left coronary artery with calcification and thrombosis of the narrowed lumen 3 cm. from the orifice. In the circumflex branch there was apparent occlusion 0.5 cm. from its origin. The condition of the aorta was not recorded. Figure 5 *E* shows a rupture of lumen into a pocket filled with lipoid cells, above to the right; fibrin bands spreading fanlike from the point of rupture and occluding the lumen, and a large atheromatous "abscess," below.

CASE 16.—A man, aged 50, a dress designer, collapsed at work, and when the doctor arrived, within five minutes, was pronounced dead. There was no history of illness. The heart weighed 330 Gm. In the descending branch of the left coronary artery, beginning 1 cm. from the orifice, was a clot extending 1 cm. Patches of concentric and eccentric thickening occurred in all of the coronary vessels, with small but patent lumens. Regions of yellow opaque thickening occurred in the arch and the thoracic portions of the aorta. In the abdominal portion of the vessel opaque yellow regions and gray scars with some calcified plaques were almost continuous. Figure 5 *F* shows a rupture of a massive atheromatous "abscess" occupying most of the vessel through a tear (left) in its thin wall into the compressed lumen, which is to the left and above.

CASE 17.—A man, aged 62, was a concessionaire in a baseball park. He had an attack of angina one year before death. He was overweight. He had lost 18 pounds (8.2 Kg.) recently on advice of a doctor. He worked hard the day before death, and ate a heavy evening meal. He was found dead in the morning. The heart weighed 500 Gm. There was marked concentric thickening of the descending branch of the left coronary artery. The circumflex branch was narrowed apparently to obliteration about 3 cm. from its origin and contained clot beyond that region. In the right coronary there was eccentric thickening of the wall with an apparently double lumen at one point containing clot. There was some thinning with myocardial atrophy and scarring over the right wall of the left ventricle extending onto the septum, as well as atrophy of the right papillary muscle. In the thoracic portion of the aorta occurred large, raised atheromatous patches, the largest 3.5 cm. in diameter. In the abdominal portion of the vessel were yellow opaque streaks above and larger raised yellow patches below, the area above the bifurcation being relatively free. Figure 5 *G* shows four atheromatous foci. The lumen in the center is seen filled with a thrombus and material from an atheromatous cavity.

CASE 18.—A man, aged 66, a painter, was arrested for drunkenness. He had made no complaints of illness. He talked with a fellow prisoner thirty minutes before he was found lying on the cot in his cell, dead. The heart weighed 290 Gm. There was narrowing of the descending branch of the left coronary artery, with almost complete obstruction 2 cm. from the orifice. The wall was gray and opaque. The vessel below was filled with clot. In the right coronary artery

no gross evidence of sclerosis was found. Figure 5H shows *atheromatous "abscesses" on both sides of the central lumen, which contains atheromatous material below and a thrombus above.*

CASE 19.—This was an instance of coronary insufficiency. A man, 60 years of age, a waiter, collapsed while working in a lunchroom and died immediately. He had been in apparently good health, save for complaints about his teeth. The heart weighed 380 Gm. Small gray fibrous regions were observed in the

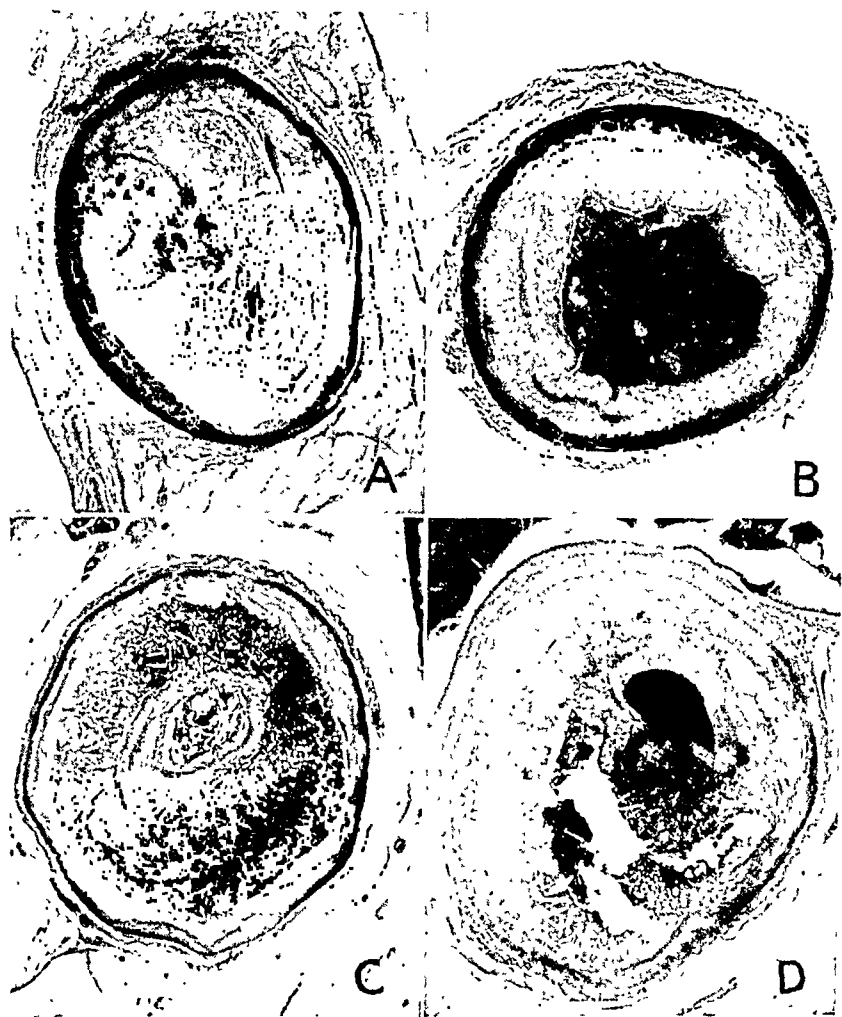


Fig. 6.—Exceptions in group 2. Atheromatosis in young men. *A* (case 20), organized thrombus in lumen; atheromatous "abscess" occupying most of vessel. *B* (same case), terminal thrombosis in fibrosed vessel. *C* (case 21), canalized lumen surrounded by atheroma. *D* (same case), terminal rupture of vessel into atheromatous "abscess."

myocardium near the apex and along the junction of the wall and the septum in the region of distribution of the left coronary artery. The orifice of the right coronary artery was small. The descending branch of the left coronary artery was almost completely obliterated except for a pinpoint-sized opening about 2 cm. from the orifice, with some calcification. The lumen was generally somewhat narrowed. The circumflex branch was of relatively large caliber, with patches

or thickening of the walls. The right coronary artery was rather small, with marked thickening of the wall and narrowing of the lumen 1.5 cm. from the orifice. The aortic sclerosis was advanced, of nodular type with scarring in the arch and thoracic regions together with plaques of lime salts, and with many high atheromatous foci in the abdominal portion of the vessel. Figure 5 I shows a large atheromatous process almost surrounding the lumen, which is very small.

#### EXCEPTIONS IN GROUP 2 (FIG. 6)

CASE 20.—A man, aged 37, an instructor in an automobile school, had been well except for attacks of indigestion. He had been an intermittent drinker. On his return from overseeing the registration of a student, he said that he did not feel well about the heart and had had to vomit at the registry. He went out to a restaurant, sat down in a chair and collapsed, dying immediately. The heart weighed 370 Gm. There was hypertrophy of the left ventricle. No infarction was seen. In the left coronary artery, beginning 0.6 cm. from the orifice, was a clot which extended 1 cm. Below this the wall of the vessel was thickened and almost completely obliterated for 1.5 cm. In the circumflex branch were found minor yellow opaque patches of thickening. In the midregion of the right coronary artery the wall was thickened almost to obliteration. The aorta was not described. Figure 6 A shows a very large atheromatous focus, but the site of the lumen (left) is occupied by organizing tissue replacing evidently a thrombus. In a section at a higher level (B) the thrombus which caused death in a second attack is shown.

CASE 21.—A man, 38 years of age, a veteran being compensated for a cardiac condition, collapsed while wading in shallow water. Autopsy disclosed no evidence of drowning. The heart weighed 610 Gm. An old infarction and thinning of the septum and adjacent left ventricular wall running upward from the apex 8.5 cm. and 5 cm. wide were found. The descending branch of the left coronary artery was grossly occluded 2 cm. from the orifice, with some calcification. The circumflex branch was also occluded 2 cm. from the origin. Beyond these regions of occlusion the vessel was filled with blood. In the right coronary artery there was thickening of the wall with a small lumen free from clot. In the aorta were found a series of small white scars in the descending arch and about the orifices of the branches, notably the intercostal. Calcified plaques occurred in the upper abdominal part of the vessel with yellow opaque deposits scattered in the part below. Figure 6 C shows a large atheromatous focus encircling a central fibrous layer about what was the lumen. At the site of the lumen occur a series of canaliculi, apparently resulting from an old organized thrombus. At a different level (D) there is rupture from the vascular lumen into an atheromatous pocket.

It is evident in each of these exceptions to the rule, occurring in young men, that earlier thrombosis was responsible for the older age reaction shown.

#### SUMMARY OF OBSERVATIONS IN GROUP 2

The formation of an atheromatous cavity or "abscess" begins by the laying down of masses of lipoid cells with a minimal amount of supporting fibrous tissue. The partitions between the cells in many cases are slender strands of reticulum (fig. 7 A). These massive accumulations of cells are dependent on imbibition for their nutrition, even when, in some cases, vasa vasorum have penetrated through the media. As



Fig. 7.—Human coronary arteries showing evolution of atheromatous "abscess:"  
*A*, lipid cell masses near lumen;  $\times 110$ . *B*, detail of lipid cell masses;  $\times 175$ .  
*C*, necrotic lipid cells with splitting of lipid esters;  $\times 200$ . *D*, atheromatous  
 "abscess;"  $\times 76$ .

a result of the hazard to the nutritive supply, necrosis tends to occur (fig. 7 *C*). This is associated with the splitting of the esters contained within the cells, and cholesterol crystals are formed. The space occupied by the dead cells is converted into a cavity filled with living and necrotic cells, detritus, cholesterol crystals and fluid (fig. 7 *D*). Such an accumulation was given the name of atheromatous "abscess" from its gross appearance. The lesion is not, however, inflammatory in any sense. Striking is the constant absence of bacteria and of inflammatory cells from such a focus. The protective properties of cholesterol are probably responsible for this constant absence of infection.

The standard cause of death in this group is the rupture of an atheromatous cavity into the lumen, secondary thrombus formation occurring in most of the instances (cases 13, 15, 16, 17 and 18; fig. 5 *C*, *E*, *F*, *G* and *H*). Or rupture may occur from the lumen into an atheromatous focus (case 14; fig. 5 *D*). Death may also arise from coronary insufficiency (case 19; fig. 5 *I*).

#### CONCLUSIONS

From this study of coronary sclerosis certain facts appear: 1. Atherosclerosis is a disease and not the inevitable consequence of age, since it appears in the young and may be highly selective in its localization. 2. The characteristic lesion in youth is a fibrosis associated with the presence of lipid cells, which do not accumulate in large aggregations, because of the growth of the fibrous tissue. 3. The characteristic lesion at older ages is the accumulation of large collections of lipid cells with minimal connective tissue support. As a result of poor nutrition, massive necrosis occurs, giving rise to so-called atheromatous "abscesses." 4. The standard cause of death in the young is thrombosis following subendothelial necrosis which extends to the endothelium. 5. The standard cause of death in the older group is the rupture of an atheromatous "abscess" into the lumen, usually followed by thrombosis. 6. The process is primary in the intima; stresses favor the localization of the lesions; morphologic lesions in the elastica and media occur secondarily. 7. The disease is not inflammatory in origin.

## II. EXPERIMENTAL ATHEROSCLEROSIS IN THE RABBIT

### SUITABILITY OF RABBITS FOR THE PRODUCTION OF EXPERIMENTAL ATHEROSCLEROSIS

In the period from 1903, when Josué<sup>4</sup> claimed to have produced medial arteriosclerosis in rabbits by the use of epinephrine, up to 1912, a series of papers was published by various authors describing the

4. Josué, O.: *Compt. rend. Soc. de biol.* 55:1374, 1903.

occurrence of spontaneous sclerosis in the aortae of normal rabbits, the highest occurrence being 34.6 per cent recorded by Miles and Johnstone,<sup>5</sup> and 52 per cent, by Levin and Larkin.<sup>6</sup> The lesions described, however, were almost without exception of medial origin or limited to the media, and Miles and Johnstone's photomicrographs, all of medial lesions, show calcification in 3 out of 4 illustrations, while Levin and Larkin's<sup>7</sup> illustrations show medial lesions and calcification in 3 of 5 normal animals. These statistics were used to controvert claims that the type of sclerosis appearing in the media in experiments with epinephrine was due to the epinephrine used. Levin and Larkin concluded: "It would seem, therefore, that the rabbit is not a suitable animal for the study of experimental arteriosclerosis."

The only resemblance of the arteriosclerotic lesions described by Miles and Johnstone and Levin and Larkin, or produced by epinephrine, to any form of human arteriosclerosis is to the so-called Mönckeberg type found in the peripheral arteries and rarely in the aorta or visceral arteries. Mönckeberg regards this as independent of true arteriosclerosis, since there are slight changes in the intima and extreme changes in the media. It can be agreed that for the experimental production of this type of sclerosis the rabbit is not a suitable animal.

In viosterol sclerosis, the viosterol apparently serves as a menstruum only, the radiant energy which it carries being responsible for the arterial lesions. These lesions are medial, as are the spontaneous and epinephrine-caused scleroses discussed, and have little or no relation to those of human atherosclerosis.

The common type of human arteriosclerosis is *atherosclerosis*, which makes up at least 95 per cent of sclerotic lesions of human visceral arteries (syphilis excluded), and is primarily an intimal process, independent of the uncommon Mönckeberg type. The intimal form of sclerosis is rare in younger rabbits, such as are used for laboratory work. Clarkson and Newburgh<sup>8</sup> cited records of 3,024 rabbits reported on by several workers in experimental medicine with 10 possible examples (0.33 per cent) of spontaneous sclerosis of the aorta which might be of the atherosclerotic type. If by experimental methods one can produce over 90 per cent of positive atherosclerotic results, it is evident that discussion of the possibility of spontaneous processes invalidating the results is futile. Moreover, if lesions are produced which cover a large part of the aorta in contrast to the relatively minute lesions described so rarely in normal animals, the importance of possible spontaneous lesions becomes minimal.

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5. Miles, A. B., and Johnstone, O. P.: J. A. M. A. **49**:1173, 1907.

6. Levin, I., and Larkin, J. H.: Proc. Soc. Exper. Biol. & Med. **7**:109, 1909.

7. Levin, I., and Larkin, J. H.: J. Exper. Med. **13**:24, 1911.

8. Clarkson, S., and Newburgh, L. H.: Arch. Int. Med. **31**:653, 1923.

*Age to Which Rabbits Live.*—The natural longevity of rabbits is a subject on which exact data are difficult to obtain. Breeders continue to use animals up to 6 years of age and even beyond, but their records are often unsatisfactory. Inquiry at biologic institutes brought no data except the record of a single animal, which was killed because senile, at 8½ years, by Dr. William E. Castle of the Bussey Institute of Harvard University. The animals used in the present research had reached a maximum age of 17 months when killed. There is an apparent relation between the single years of a rabbit's life and the decades of a human life. Viewed from this standpoint our oldest animals were comparatively only in their "teens."

#### CHOLESTEROL

According to Schoenheimer,<sup>9</sup> who has carried out extensive studies on the sterols, cholesterol is one of the most complicated substances in the animal body. Apart from ergosterol, which is found in small amounts, it is the only sterol found in the animal body, and does not occur in nature except in animal bodies. It is a relatively insoluble substance which is highly resistant to the action of enzymes, whether of bacterial, vegetable or animal origin, and is the only sterol (in addition to viosterol) which is absorbable. The many plant sterols, most of which have the same chemical formula as cholesterol,  $C_{27}H_{46}O$ , differ from it in molecular arrangement. Their nonabsorbability is apparently dependent on these differences. The sterols unite with fatty acids to form esters and tend to be associated with the glycerol fats. In mixtures with fats and in extracts of tissues by fat solvents the sterols are found in the unsaponifiable residue. Cholesterol is synthesized by the animal body, notably among the herbivora, but the principal human provision of this material comes from ingestion of the products of herbivora. Egg, milk and pork fats are the main sources of supply.

Cholesterol is present in every animal cell. Of its cell functions Starling<sup>10</sup> said: "In view of the great stability of this substance when exposed to the ordinary mechanisms of chemical change in the body, it seems probable that the part played by cholesterol is that of a framework or skeleton, in the interstices of which the more labile of the constituents of the protoplasm undergo the cycle of changes which make up the phenomena of life." The cholesterol which is secreted with the bile assists in the absorption of fats from the intestine and is itself largely resorbed. Cholesterol is believed to be responsible for the impermeability of cells to certain toxic agents, is the important agent resisting hemolysis, and is supposed to confer protective properties on cells.

9. Schoenheimer, R.: Science **74**:580, 1931.

10. Starling, E. H.: Principles of Physiology, Philadelphia, Lea & Febiger, 1930, p. 42.



That it highly resists infection is manifest in the constant absence of infection from so-called atheromatous "abscesses." It tends to accumulate in the animal body, i. e., to be stored, and its avenues of excretion are limited. Its metabolism appears to be influenced by thyroid secretion.

All animals must have a metabolic system caring for the absorption and distribution of cholesterol to satisfy the requirements of their cells, and the herbivora at least synthesize the substance. Since herbivora do not naturally ingest cholesterol their threshold of tolerance is low. It is therefore possible to overload their cholesterol metabolic systems more readily than is true with the carnivora, whose diet includes cholesterol. The limitations of time demand the experimental production, in a short period, of lesions that require a lifetime for natural production.

#### EXPERIMENTAL METHODS AND RESULTS

The experiments reported here were started with rabbits 5 months old, some of which were kept in reserve for replacements. Cholesterol was fed by catheter for a maximum period of seven months. Some rabbits died before the end of the feeding period. At the end of this period all of the surviving animals except 4 were killed. These were allowed to live for five months after feeding ceased. For the first four months of the feeding period starch paste was used as a menstruum, the pure cholesterol being finely powdered before suspension. For the remaining three months sunflower seed oil was substituted as a menstruum, the cholesterol being dissolved (1 Gm. to 20 cc.) in warm oil. The dosage was gradually increased from 0.2 Gm. to a maximum of 1.4 Gm. daily, six times a week, with intermissions if animals lost weight or were ill. A limit of tolerance at about 1 Gm. of cholesterol per diem was established. Beyond this animals tended to develop diarrhea and roughness of coat, and some died. Control animals receiving equal amounts of sunflower seed oil without cholesterol were not affected.

The results in cholesterol-fed animals were, in general, more marked than the lesions described in the literature. As measured by aortic manifestations they were as follows:

Four rabbits exhibited advanced atherosclerosis throughout the aorta with extension to its branches. Of these, 1 had been allowed to live five months following cholesterol feeding.

In 8 rabbits there were continuous lesions throughout the arch with extension to its branches and through the thoracic portion of the vessel, and there were scattered lesions below this portion. Of these, 1 had lived five months following cholesterol feeding.

In 7 rabbits continuous lesions were present in the arch with scattered foci below. Two of these had lived five months following cholesterol feeding.

In 6 rabbits there were no continuous lesions, but scattered small lesions of atherosclerosis were found.

In 2 rabbits which were killed during the period of starch and cholesterol feeding, the aortae were normal.

Five normal controls fed starch paste and later oil, 5 controls receiving insulin and 4 controls receiving alcohol showed normal aortae. In an alcohol-fed control there were 2 *medial* foci of sclerosis in the arch, 0.3 cm. in diameter.

One rabbit killed for observation in the early period, when starch paste was used as a menstruum, died at too early a date for the development of sclerosis. Even including this animal, in 25 of 27 rabbits fed cholesterol, or 92.6 per cent, atherosclerosis was produced. In 14 controls no atherosclerosis was found. In 1 control medial sclerosis was present.

Two interesting elements appear in these results. Though large amounts of cholesterol were fed equally to many animals, the gross end-results varied from small scattered lesions to extreme processes that included almost the whole of the aorta, parts of its branches, and visceral arteries, and were associated with evident hypertrophy of the heart. This variation in susceptibility is perhaps comparable to the idiosyncrasy toward atherosclerosis exhibited by human beings, who may die in the twenties from coronary sclerosis or may live to the age of 70 or 80 and show little arteriosclerosis. The absence of atherosclerosis in controls that received sunflower seed oil (without cholesterol) in equal amounts with cholesterol-fed animals is in accordance with the finding of Schoenheimer and his associates that phytosterols are not absorbable.

#### THE LESIONS

It should be kept in mind that the experimental lesions present, in general, earlier stages in the process than are seen in most human material. However, progress in the study of arteriosclerosis, as is true in other diseases, will come from the reproduction and observation of early lesions. The illustrations from experimental lesions in figures 8 to 14 inclusive are from various vessels, as indicated.

*Imbibition of Lipoid Esters.*—The first manifestation of vascular lipoidosis is the appearance of the lipoid in spaces beneath the endothelium of the blood vessels. Whether this is properly a matter of imbibition or the result of secretory activity of the endothelial cells is not known. The endothelium gives no evidence of disturbance, but is

lifted mechanically as the deposit occurs, together with the inner elastic lamina in some vessels. Some observers have reported the presence of fine droplets of lipid in the endothelial cells before the appearance of cholesterol beneath the endothelium. Figure 8 *A* is a cross-section of a

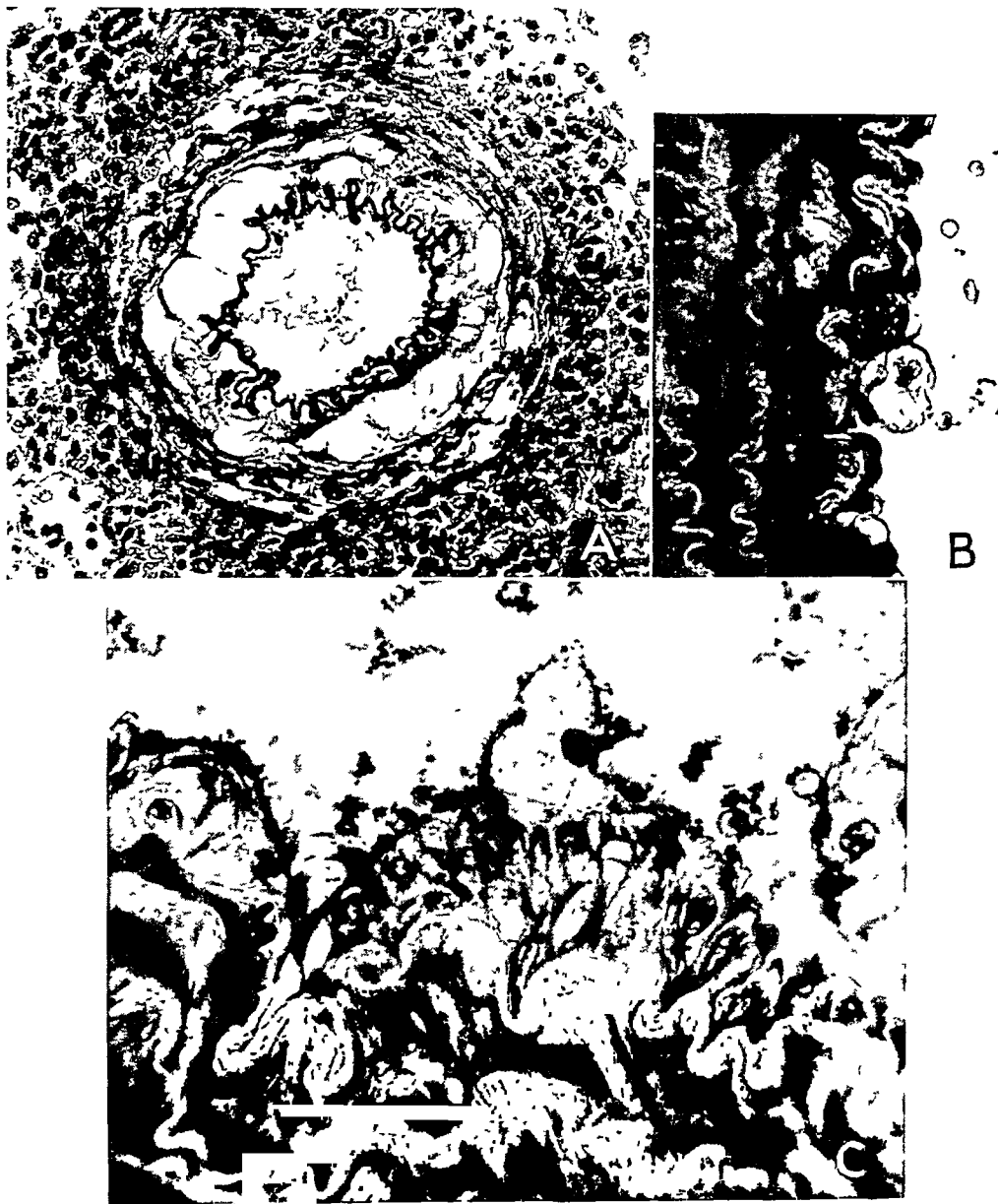


Fig. 8.—*A*, splenic arteriole of rabbit;  $\times 300$ ; lipoidosis. *B*, innominate artery of rabbit; lipoid cell adhering to wall. *C*, carotid artery of rabbit;  $\times 350$ ; lipoid macrophage invading (?) wall. Note proliferation of subendothelial connective tissue at this point.

splenic artery, in which the internal elastic lamina is seen to be carried up with the endothelial layer.

*The Macrophage—The Lipoid Cell.*—Early in the evolution of the vascular processes appear cells which engulf the cholesterol esters and give rise to the most characteristic element in human or in experimental sclerosis, i. e., the large macrophage loaded with lipoid esters in fine or coarse droplets. The source of these cells is in question. They are not resting wandering tissue cells (histiocytes) which emigrate to the place where foreign material has accumulated. They either arise locally from the subendothelial layer or are invaders from the blood stream (monocytes). Figure 8 *B* shows one of these cells attached to the wall of an innominate artery at a point where there is little evidence



Fig. 9.—Carotid artery of rabbit;  $\times 140$ ; festooned masses of subendothelial connective tissue cells enclosing lipid macrophages.

of lipoidosis. In figure 8 *C* a large macrophage is apparently invading or leaving the wall at a point where lipoidosis is beginning. These cells are found locally, in the blood stream and in the splenic sinuses.

*Massing of Lipoid Cells.*—As the disease progresses the lipid cells become massed in the subendothelial connective tissue. In the experimental form the layers of cells may be multiplied into hillocks which distort the lumen of the vessel. The cells are separated by delicate partitions of connective tissue, though more than one cell may occupy a compartment in the connective tissue. The picture when cell groups are excessive in the rapid early experimental process is shown in figure 9. The slower standard experimental lesion is illustrated in figure 10 *A*. The cells are large globular structures, and sections are unlikely to

include the nucleus in each cell of a group. Figure 10 *B* shows lipid cells grouped beneath the endothelium in a human coronary artery.

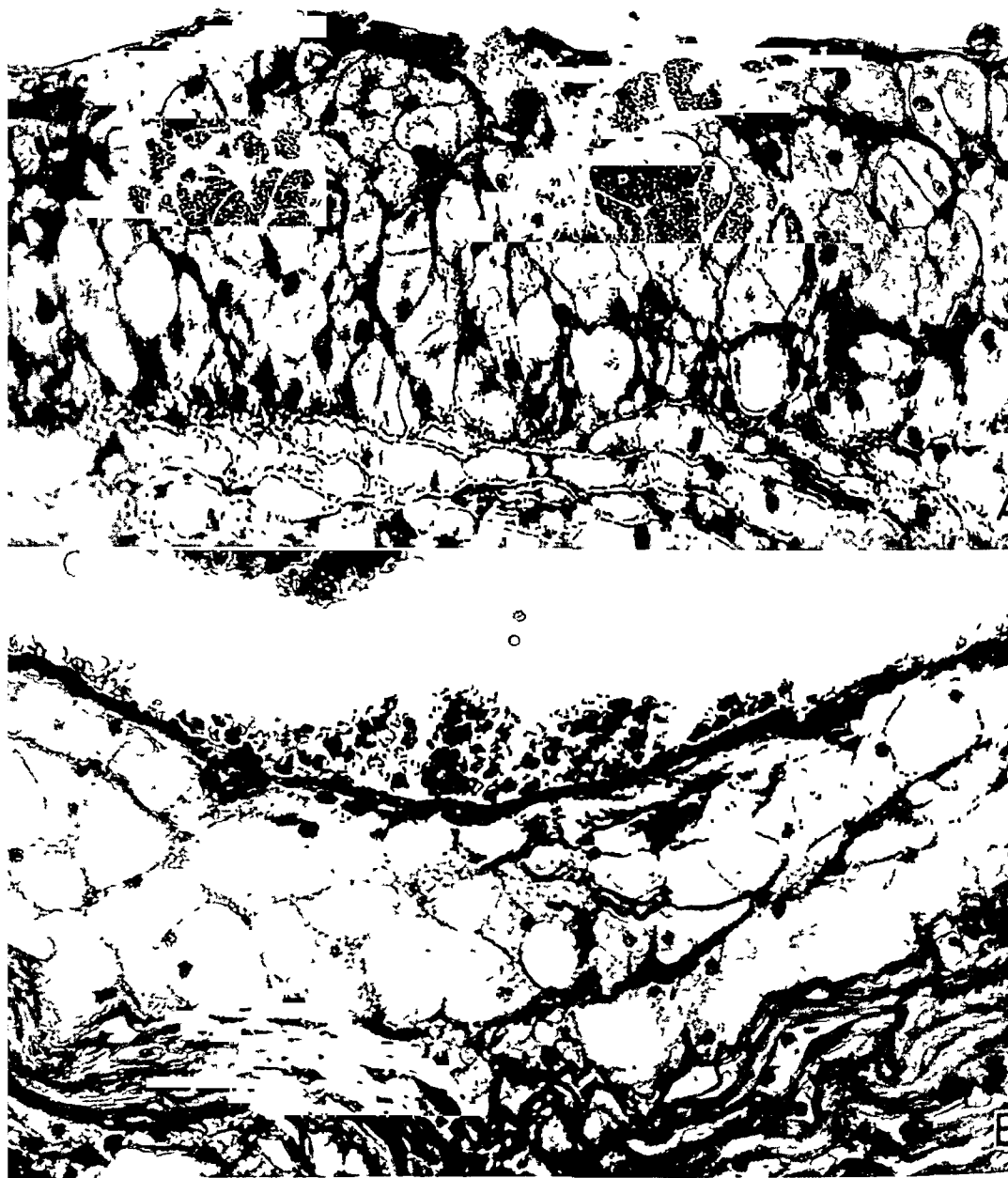


Fig. 10—*A*, innominate artery of rabbit,  $\times 300$ ; subendothelial masses of lipid cells. *B*, human coronary artery;  $\times 350$ , lipid cells beneath endothelium.

*Deep Invasion by Lipoid Cells.*—As the process ages it is common to find deeper invasion by some of the lipid cells as fibrosis of the

inner layers takes place. This is illustrated in figure 11 *A*, while figure 11 *B* shows focal invasion of the media in a human coronary artery.

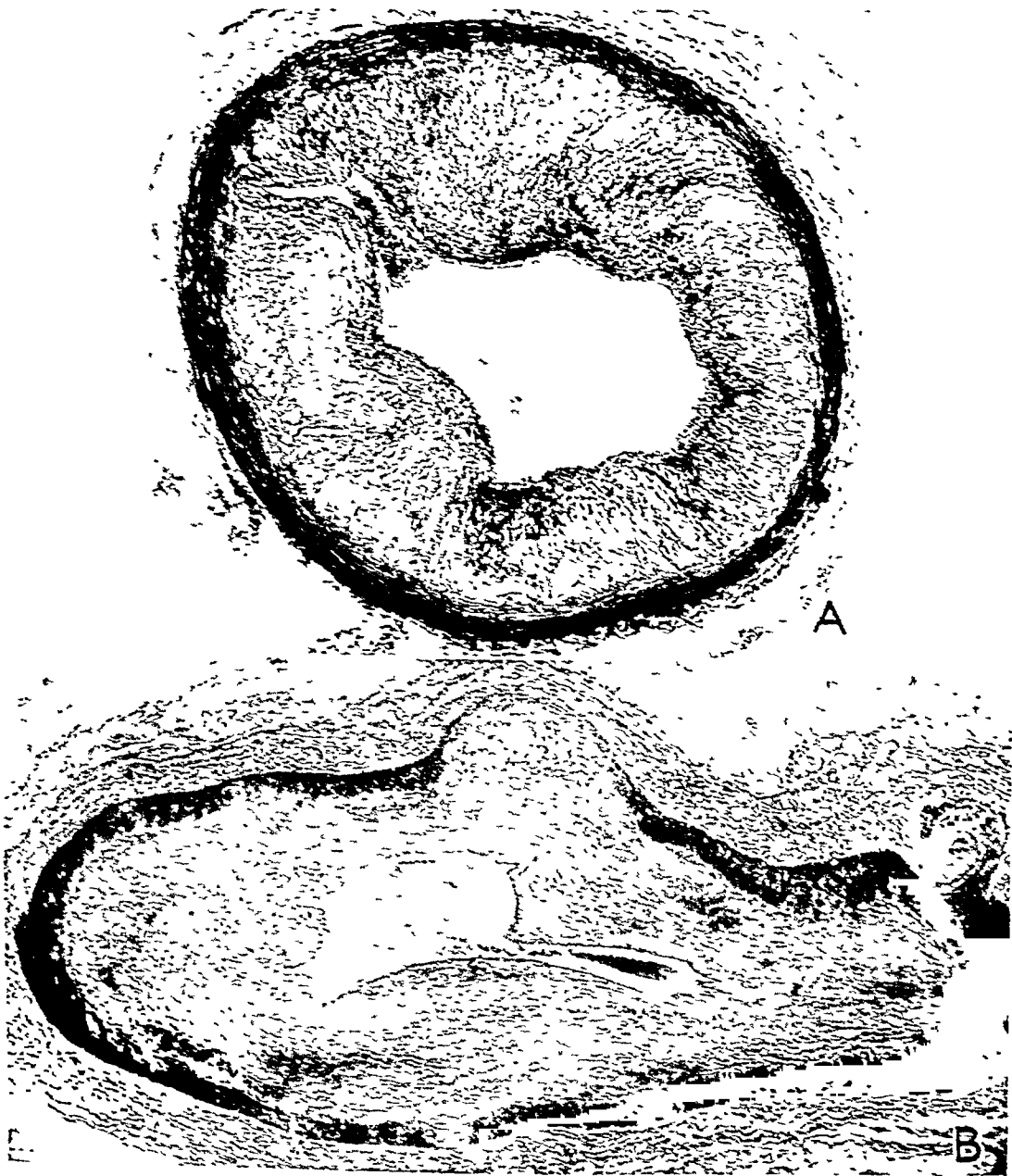


Fig. 11.—*A*, carotid artery of rabbit:  $\times 50$ ; internal fibrosis; deeper, lipid cells. *B*, human coronary artery:  $\times 23$ ; focal invasion of media by lipid cells.

*Splitting of Lipoid Esters.*—When lipid cells accumulate, even in isolated groups, necrosis of the cells tends to occur. The early stages are marked by the breaking down of the cytoplasm and the fusing of the esters, which have existed in fine droplets, into larger drops. This

is followed by the splitting of the esters and the freeing of cholesterol in crystal form. This picture is found in the rabbit lesions usually in regions where larger accumulations of lipoid cells are undergoing



Fig. 12.—Innominate artery of rabbit;  $\times 100$ ; atheromatous "abscess."

fibrosis, and in advance of the growing fibroblastic tissue. It is not a very common finding. In human coronary arteries in the young, deposits of cholesterol crystals are met with, usually in small groups, where small isolated collections of lipoid cells have undergone necrosis.

A rare lesion in the rabbit is the atherosclerotic cavity or "abscess." Antischkow has never produced it and it is not reported in the literature. It probably resulted in my series from the introduction of very large

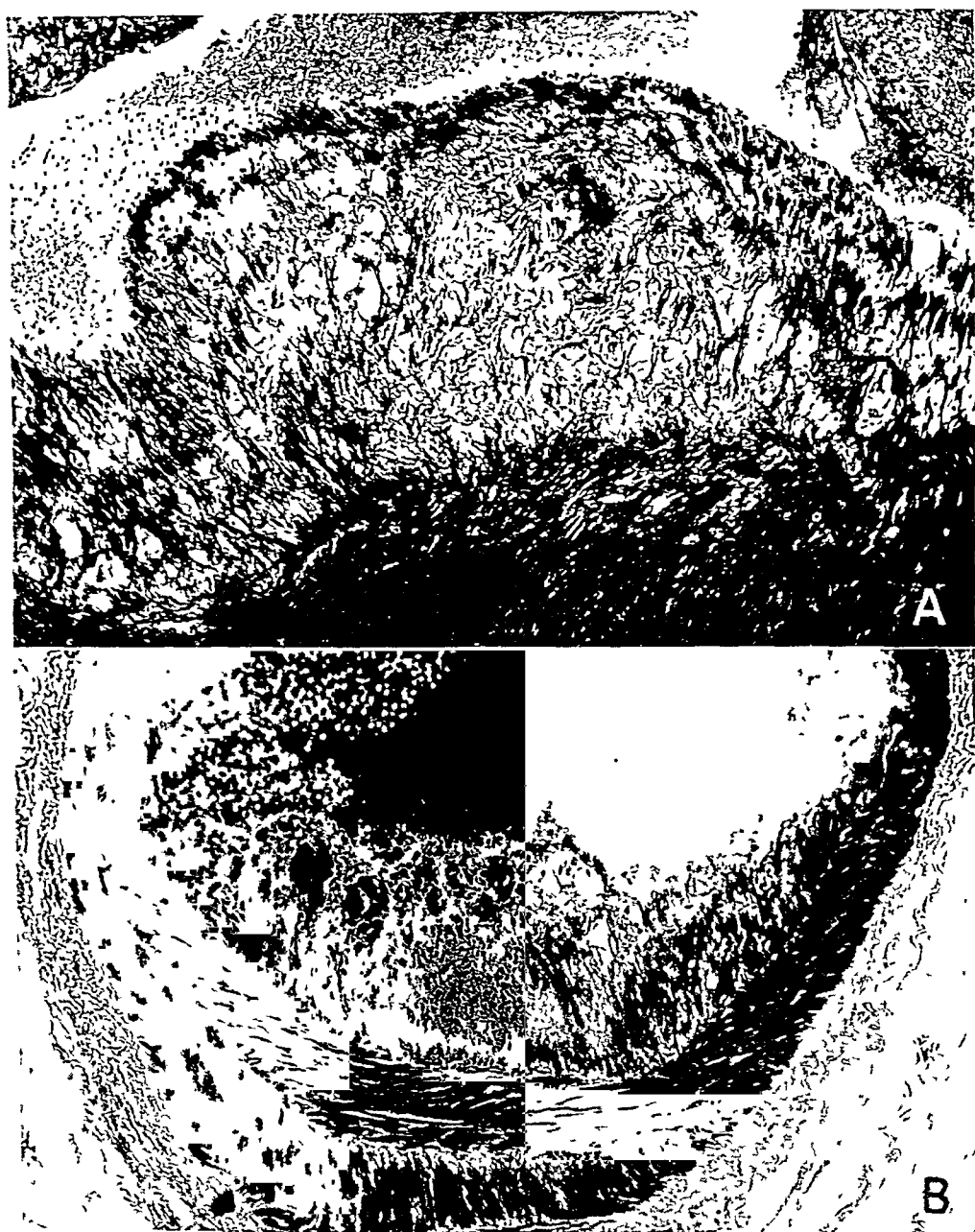


Fig. 13.—*A*, innominate artery of rabbit;  $\times 100$ ; beginning fibrosis in mid-portion of nodule of lipoid cells, before formation of dense fibrous tissue. *B*, human coronary artery in case 3;  $\times 130$ ; fibrosis replacing lipoid cells.

amounts of cholesterol into a susceptible animal. Its appearance (figure 12) corresponds closely, on a smaller scale, to the human lesion illustrated in figure 7 *D*.





Fig. 14.—*A*, subclavian artery of rabbit;  $\times 50$ ; eccentric fibrosis. *B*, subclavian artery of rabbit;  $\times 21$ ; concentric fibrosis.

*Fibrosis.*—The standard progressive change in the rabbit is fibrosis. When the accumulation of lipid cells has reached a certain degree, young fibroblasts appear and replace the cells. This replacement is total, including the lipid cells, their contents and the supporting tissue. It is unaccompanied in general by the splitting of the esters, though occasionally small deposits of cholesterol crystals are found. An early stage of fibrosis is seen in figure 13 *A*. A central island of fibroblastic tissue is invading and replacing the new lipid tissue. Figure 13 *B*, a comparable picture, is from a branch of the human left coronary artery in case 3. The human lesion is older, and the mass of tissue in the center is fibrous, but fibroblastic cells are active on the sides.

In both rabbit and man the formation of fibrous tissue is more frequently diffuse than local, the delicate strands between the lipid cells becoming thicker as the lipid cells disappear.

The result of fibrosis, which tends to progress *pari passu* with the occurrence of lipid cells, is the formation of fibrous masses distorting and narrowing the lumen of the vessel. Eccentric fibrosis is illustrated in figure 14 *A* and concentric fibrosis in figure 14 *B*. The fibrous tissue in the experimental lesions that I describe was largely limited to the intima, but Wolkoff<sup>2c</sup> described, with reproductions, advanced lesions in which the fibrous tissue had invaded the media in rabbits allowed to live for years following the cessation of cholesterol feeding.

### III. COMPARISON OF HUMAN AND EXPERIMENTAL RABBIT ATHEROSCLEROSIS IN THE CORONARY ARTERIES

(All of the illustrations which follow are from coronary arteries, save figure 21.)

The lesions of human coronary sclerosis reported in part I were observed in advanced cases which terminated in death. In the secondary branches of the coronary arteries from some of these cases early lesions (e. g., fig. 13 *B*) were found. In general, however, the human process is of so slow evolution that initial or early processes are hard to find. Search for the earliest processes was finally successful in the left coronary arteries of infants who died in the early days of life from congenital heart disease. The burden thrown on the myocardium in its effort to keep up a circulation under the handicap of developmental faults causes unusual stresses on the coronary arteries, with resulting lesions. These have supplied illustrations of the earliest natural processes found.

Figure 15 *A* from the coronary artery of a rabbit illustrates lipoidosis, which is the first stage in the process. In the larger vessel, the descending branch of the left coronary artery, there is a deposit of lipid under the endothelium at one point; a more massive deposit is shown in the two branches above.

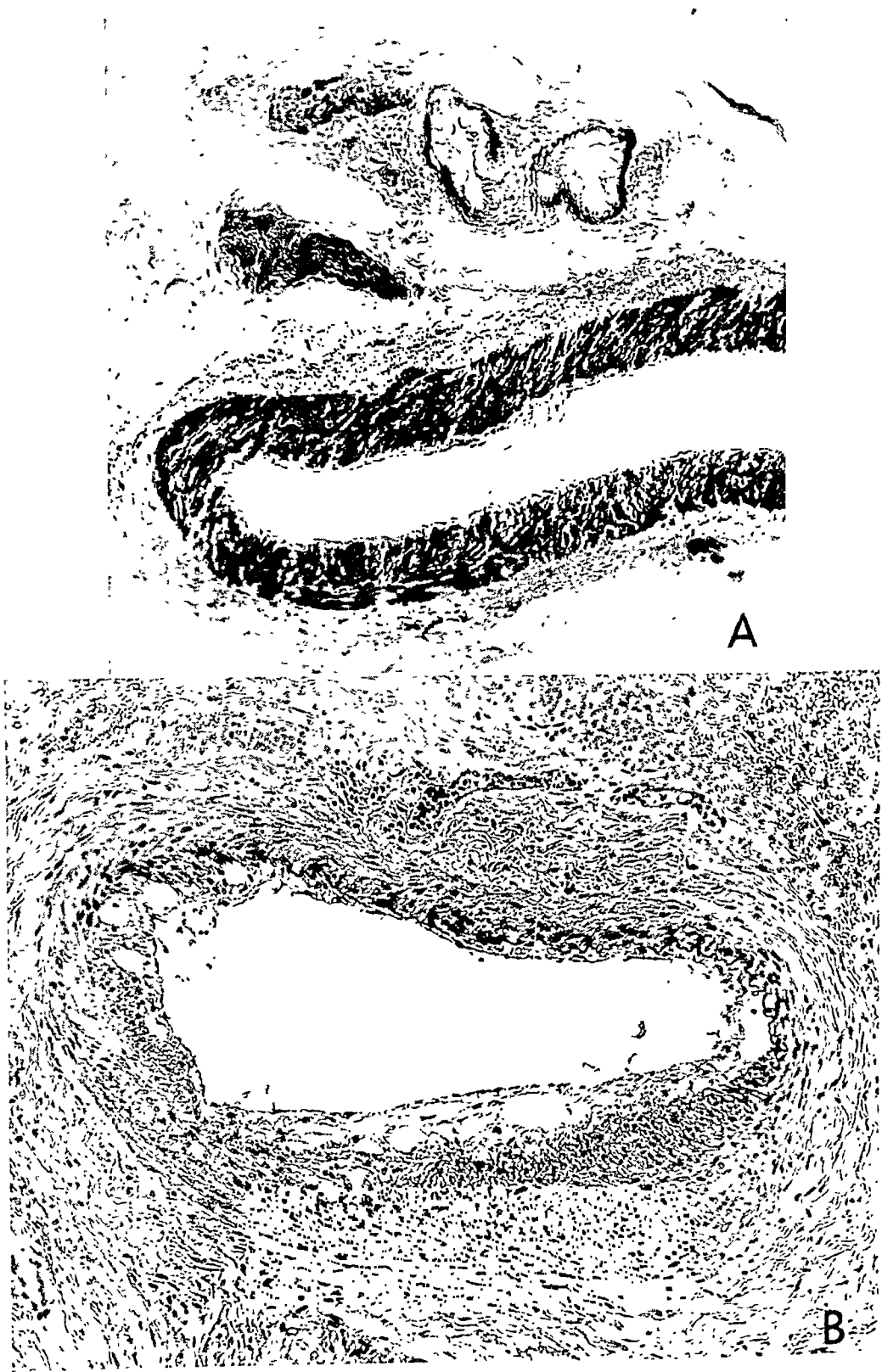


Fig. 15.—*A*, coronary artery of rabbit;  $\times 120$ ; lipoidosis, focal in main vessel and general in branches. *B*, human coronary artery at 3 days of age;  $\times 150$ ; lipoidosis and lipid cells.

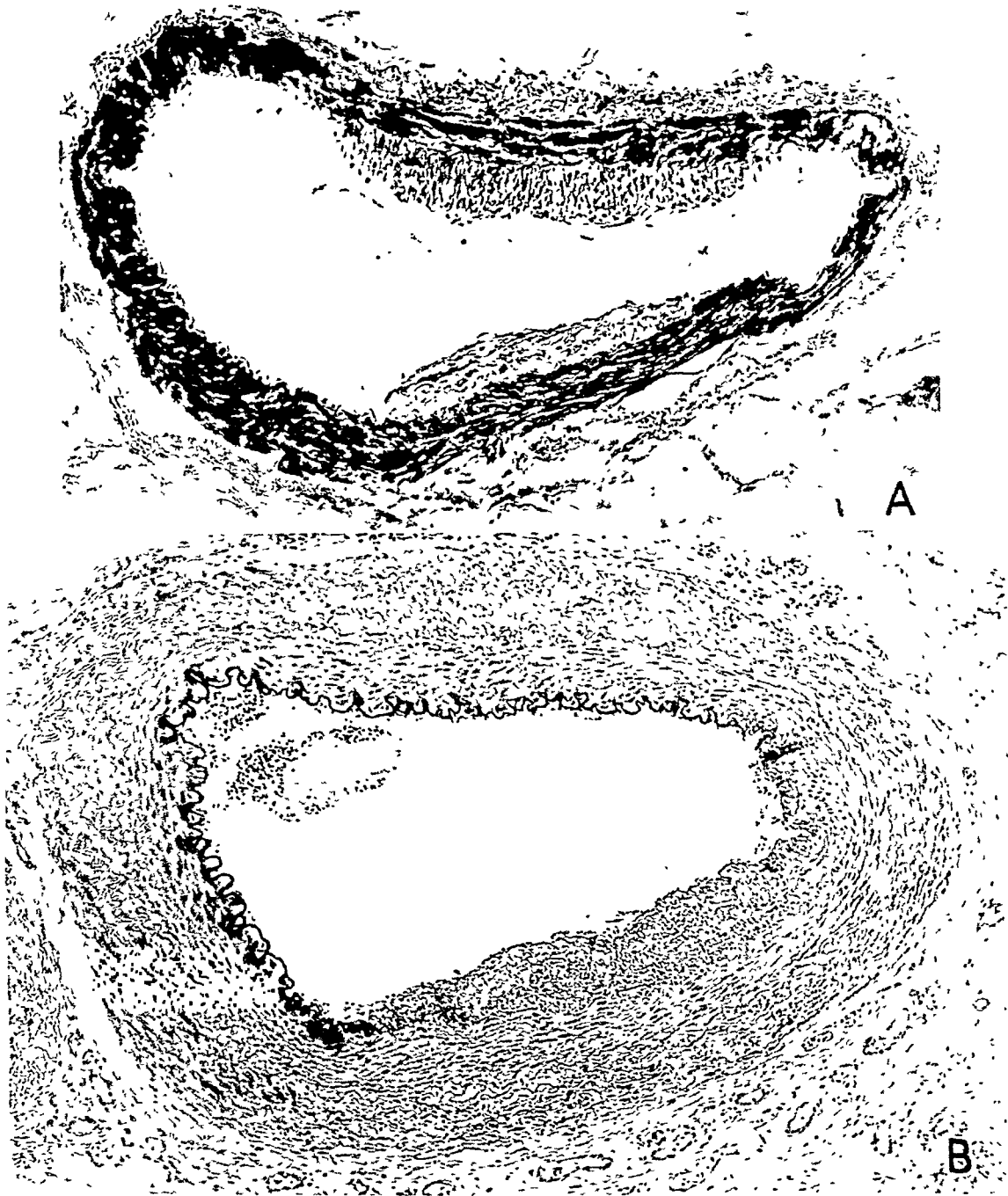


Fig. 16.—*A*, coronary artery of rabbit;  $\times 120$ ; early lesion with lipoid cells below and early fibrosis above. *B*, human coronary artery at 33 days of age;  $\times 85$ ; early fibrosis; elastica largely absent under lesion.

Figure 15 *B* is from the left coronary artery of a blue baby who died three days after birth. At postmortem examination there was found pulmonary and tricuspid atresia with cor triloculare. The vessel shows diffuse subendothelial lipoidosis, and lipid cells are present at each end of the affected region. In figure 16 *A* from the coronary artery of a rabbit two stages in the evolution of the characteristic lesion are seen. On the lower side is a relatively early process in which lipid cells are moderately abundant. On the opposite surface is a cellular



Fig. 17.—Human coronary artery at 14 years of age;  $\times 60$ ; formation of irregular buffer layer.

fibrous lesion resembling closely that seen in the buffer layer of the normal coronary artery. Figure 16 *B* is from the left coronary artery of an infant with mongolism and also an imperforate anus which was successfully operated on. Death occurred thirty-three days after birth. Postmortem examination disclosed defective auricular and ventricular septums and a patent ductus arteriosus. The figure shows a subendothelial fibrous lesion along the lower edge of the lumen. The prominent internal elastic lamina is lost under the new tissue in the right lower quadrant. Figure 17, which is from a branch of the normal left coronary artery of a boy aged 14, reveals the irregular distribution of the subendothelial tissue in the buffer layer, which has not yet reached the

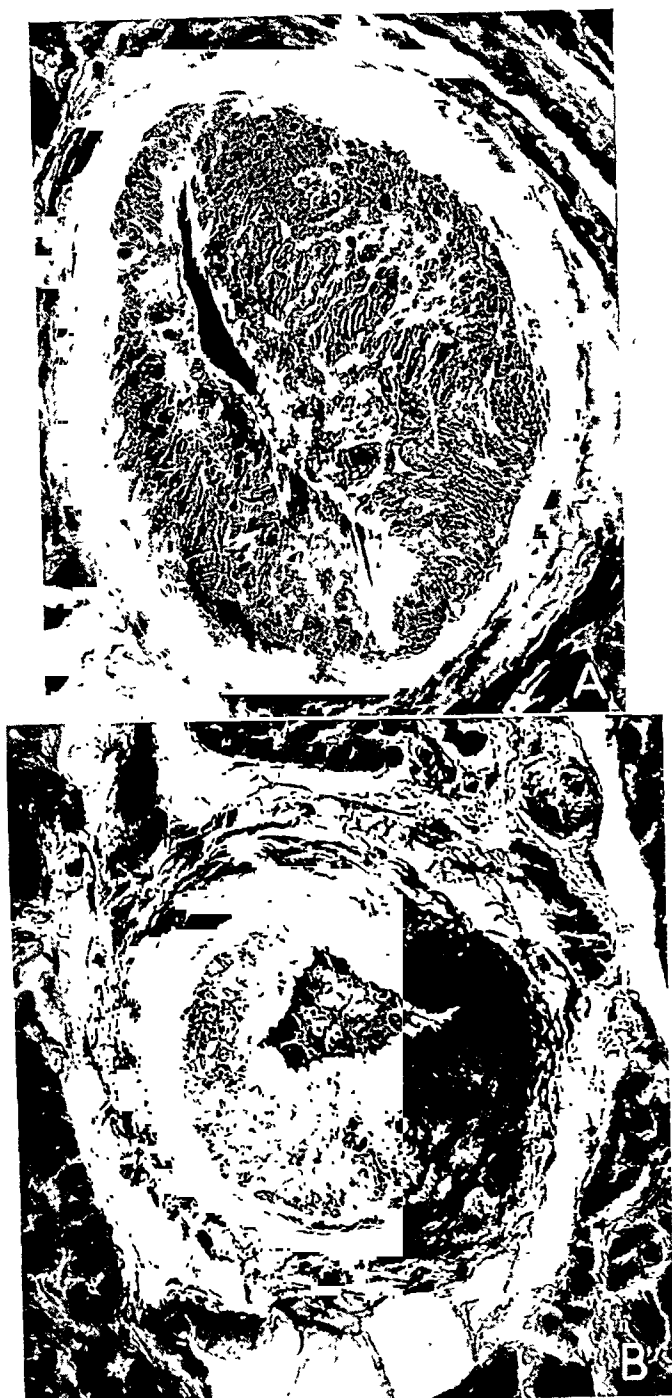


Fig. 18.—*A*, coronary artery of rabbit;  $\times 300$ ; lipoidosis with concentric fibrosis. *B*, coronary artery of rabbit;  $\times 300$ ; advanced fibrosis in small branch with intact, though flattened, elastica.

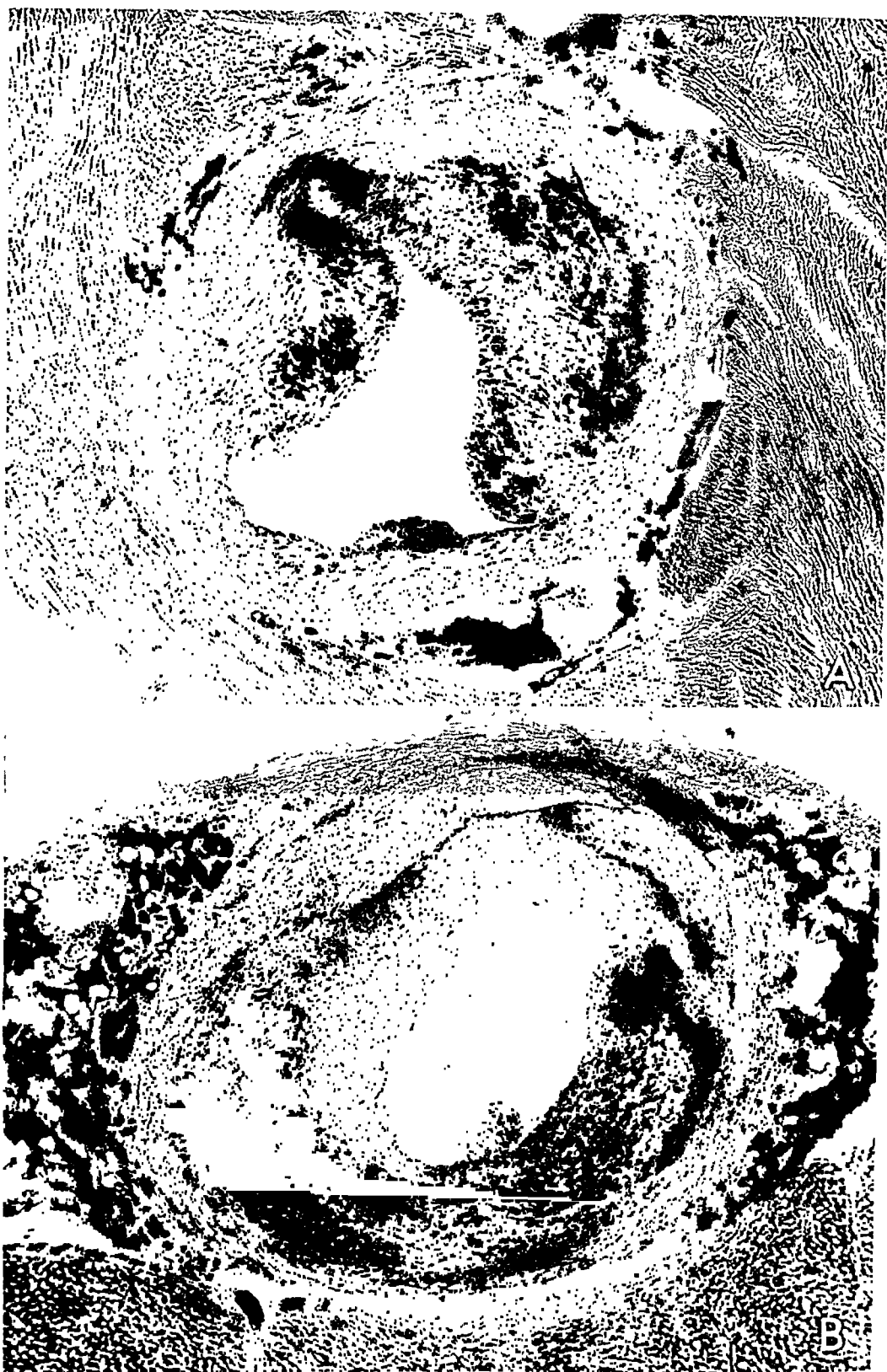


Fig. 19.—*A*, coronary artery of rabbit;  $\times 60$ ; advanced fibrosis with secondary fatty changes. *B*, human coronary artery;  $\times 30$ ; frozen section stained with sudan IV. Compare with *A*.



Fig. 20.—*A*, heart of rabbit;  $\times 17$ ; frozen section, stained with sudan IV; late lesion in main vessel; lipoidosis of branches. *B*, coronary artery of rabbit;  $\times 120$ ; eccentric fibrosis in descending branch; narrowing of lumen of auricular branch above. *C*, section of cardiac auricular wall of rabbit;  $\times 150$ ; myocardial atrophy and repair.



perfection of the layer of regular thickness found in the main vessel. The fibrous tissue making up this layer corresponds in its details with that seen in the upper lesion in figure 16 *A*. A concentric lesion producing marked narrowing of the lumen, but still rich in lipoids, may be seen in figure 18 *A* and an eccentric, more fibrous process in figure 18 *B*.

As the fibrous tissue ages secondary fatty metamorphoses arise, and necrosis of the intimal tissue may occur. In figure 19 *A* and *B* are shown frozen sections stained with sudan IV and hematoxylin. The rabbit lesion (*A*) exhibits an eccentric fibrosis with secondary fatty changes. The human lesion (*B*) is more advanced, but the fundamental character of the lesions is similar in all respects.

The cyclic character of the atherosclerotic process is suggested by the occurrence of comparatively recent lipoidosis in the smaller branches of the left coronary artery of a rabbit (fig. 20 *A*) in contrast to the advanced lesion in the main vessel, as illustrated in figure 19 *A*. This rabbit had received no cholesterol for five months before its death. The lipoidosis in the splenic arteriole seen in figure 8 *A* was from another animal which had been allowed to live five months after cholesterol feeding had ceased.

#### SECONDARY EFFECTS

A common end-result of human coronary sclerosis is focal atrophy of the heart muscle and replacement fibrosis (so-called chronic myocarditis). In figure 20 *B* fibrosis of a larger branch of the coronary artery of a rabbit is seen with marked narrowing of the auricular branch above it. Section of the auricular wall disclosed focal atrophy of the heart muscle and repair (fig. 20 *C*).

#### VARIATION IN AORTIC LESIONS

Three rabbit hearts and aortae are shown in figure 21. The central heart and aorta were obtained from a normal rabbit of certified age killed when 7 years and 1 month old, i. e., approaching senility. The aorta shows no lesions. For comparison, below it, are the heart and aorta of rabbit 29, killed when 12 months old, after being fed 128.8 Gm. of cholesterol over a period of seven months. There is a diffuse atherosclerosis of the arch with patches about the intercostal orifices and there are larger foci in the abdominal portion of the vessel. In a word, there is a mild to moderate atherosclerosis of the vessel. The upper heart and aorta are from rabbit 32, which was found dead. It had been fed 1.4 Gm. of cholesterol on the day before and had ingested a total amount of 113.8 Gm. of cholesterol. When this rabbit was found its fur was soiled with a diarrheal discharge. In the vessel

there is a massive atherosclerotic process extending continuously from the aortic ring to the lower abdominal region. That there was a difference in the ability of rabbits 29 and 32 to metabolize cholesterol there can be no question.

#### DEATH OF A RABBIT FROM CORONARY INSUFFICIENCY?

In figure 22 is reproduced a section of the aorta and the left coronary artery at its origin from rabbit 32. The aorta shows little change at this point. The contrast between the thick aortic media and the thin coronary wall is striking. There is also indicated the sharp curve which the left coronary artery takes as it leaves the aortic wall in its descending

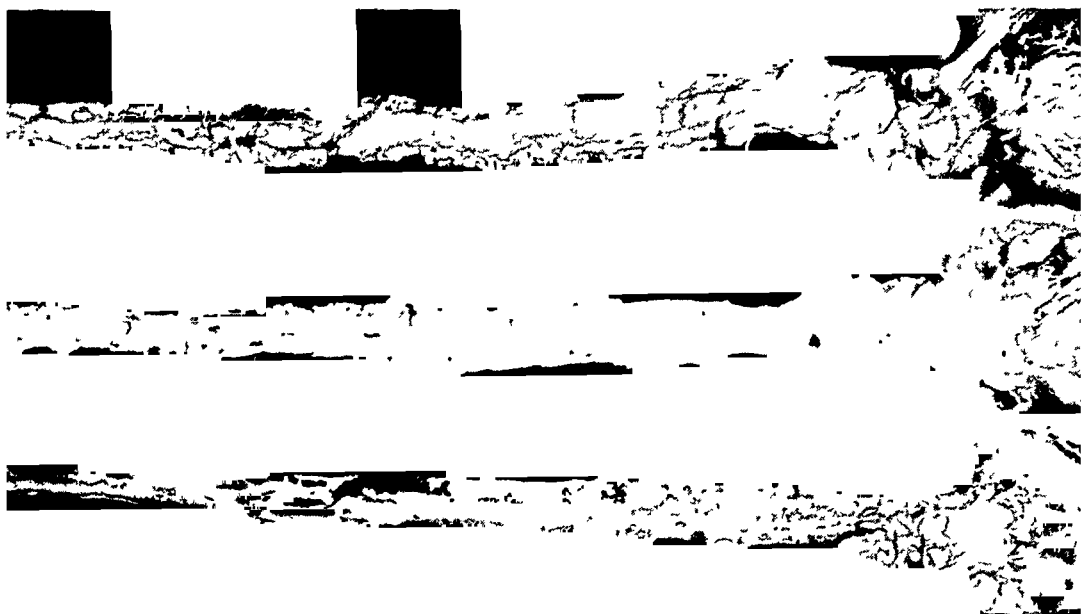


Fig. 21.—Hearts and aortae of rabbits. See text.

course. This curve is sharper than that taken by the human left coronary artery but is of similar character. The coronary lesion, still rich in lipoid cells, has narrowed the lumen of the vessel to a high degree. If a lesion of similar degree were discovered in the left coronary artery of a human being who was found dead with evidence of indigestion there is little doubt that the cause of death would be properly referred to coronary sclerosis and insufficiency.

#### CONCLUSIONS

From the comparative study of human and rabbit atherosclerosis the following conclusions are drawn: 1. The lesions of human atherosclerosis can be reproduced in the rabbit by the feeding of cholesterol. 2. The fibrosis which is characteristic of human coronary lesions in

the young is the characteristic lesion in cholesterol atherosclerosis in young rabbits. Fibrosis is therefore a reaction of youth and not of species. 3. The evolution of the lesions through lipoidosis, lipid cell formation and fibrosis, more rapidly produced in experimental animals, can be followed in greater detail in these animals than in human lesions, in which progress is so slow that the stages in the progression are distinguished with difficulty.



Fig. 22.—Aorta with left coronary artery of rabbit;  $\times 65$ .

#### COMMENT

Any metabolic agent capable of producing atherosclerosis must have been an article of diet from early times, since atherosclerosis has been found in mummies.<sup>11</sup> One thinks of cholesterol in terms of blood or bile cholesterol. This, however, is only the mobilized cholesterol. The

11. Ruffer, M. A.: *J. Path. & Bact.* **15**:451, 1911.

substance is a necessary part of every animal cell, forming, from Starling's concept, the stable groundwork of the cell cytoplasm. As far as any one knows no cholesterol is synthesized by the human body. All of the supply is ingested. The most urgent demands for it come at times of most rapid cell formation. Egg yolk is intended for the embryo. Milk is intended for the infant. It is interesting to note that Wells, in his "Outline of History," records that it was relatively late in the evolution of primitive man that he developed what Wells calls "the rather unnatural use of animal's milk as food." The high blood cholesterol found in pregnant women marks the mobilization of this substance for the needs of the fetus in utero. Man is the only animal that ingests eggs and milk throughout its lifetime. Man is also the only animal, as far as is known, which dies in early life from coronary sclerosis, and which acquires atherosclerosis almost universally in advanced life.

That cholesterol, which is so highly insoluble, is modified during metabolism by union with other lipoids is probable from the work of Kimmelstiel.<sup>12</sup> Lipoid combinations which can be shown in vitro to increase the solubility of cholesterol were isolated by him from atherosclerotic aortae. It was only where atheromatous "abscess" formation had occurred, with the precipitation of cholesterol crystals, that a very high, almost exclusive cholesterol content was found. The absorption from the intestine and distribution of cholesterol in the body apparently depend on the combination of this substance with other lipoids. The failure to produce atherosclerosis in rabbits following intravenous injection of the pure substance may be due to this factor.

As one studies human atherosclerosis the analogy of the disease to diabetes repeatedly suggests itself. It is true that sugar is one of the most soluble and combustible food substances, and that its permanent storage in the body does not occur, while cholesterol is not combustible, its avenues of excretion are limited, and it tends to be stored. However, as in diabetes, marked variation in idiosyncrasy to the substance seems to exist. As in diabetes, the inheritance of a poor cholesterol metabolism appears to be associated with the tendency to an early death from coronary sclerosis. As in diabetes, advancing age, with the development of inefficiency of the cholesterol metabolism, appears to be associated with more frequent late manifestations of the disease. Finally, as in diabetes, an internal secretion, in this case that of the thyroid gland, appears to control the experimental production of atherosclerosis.

Recent human experience in diabetes supports the experimental findings in rabbits following cholesterol feeding. During and following

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12. Kimmelstiel, P.: *Virchows Arch. f. path. Anat.* 282:402, 1931.

the period when diets excessively rich in fat were used in the treatment of diabetes there was so great an increase of atherosclerosis that Shields Warren wondered whether the increase was due to the diabetes or to the treatment of the disease. Roentgen examination of the legs of some of the children treated disclosed evidence of calcified atherosclerotic arteries, and xanthomas were common. Recent reports from the Joslin clinic indicate that under a diet of lower fat content in the treatment of diabetes xanthomas no longer appear and roentgen evidence of calcified arteries in the legs of the children is lacking.

# ABSCESSSES OF THE LIVER CAUSED BY BACTEROIDES FUNDULIFORMIS

REPORT OF TWO CASES

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Under the generic designation *Bacteroides* Castellani and Chalmers,<sup>1</sup> in 1919, brought together a rather large group of anaerobic, nonsporulating bacilli which previously had been without systematic classification. The generic name *Bacteroides* proposed by these investigators was adopted by a committee of the Society of American Bacteriologists and given a place in their systematic classification, as recorded in "Bergey's Manual of Determinative Bacteriology."<sup>2</sup> According to the manual, the characters of the genus are: "Motile and nonmotile rods, without endospores. Show good growth on ordinary culture media; without pigment formation. Obligate anaerobes." Seventeen species are listed; ten are gram-positive and seven, gram-negative. The species enumerated are described as normal inhabitants of the intestinal tract of man and as the cause of suppurative and gangrenous infection in man.

Veillon and Zuber,<sup>3</sup> in 1898, were apparently the first to describe anaerobic organisms of this type and to point out their importance. They obtained several species from cases of appendicitis and other suppurative conditions, to which they gave the name *Bacillus ramosus*, *Bacillus serpens*, *Bacillus fragilis*, *Bacillus furcosus* and *Bacillus fusiformis*. Hallé,<sup>4</sup> at about the same time, confirmed this work by finding similar organisms in the vaginal flora and in suppurative conditions about the genital tract of the female. He introduced a new species,

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From the Department of Pathologic Anatomy, the Mayo Clinic.

1. Castellani, Aldo, and Chalmers, A. J.: *Manual of Tropical Medicine*, ed. 3, London, Baillière, Tindall & Cox, 1919.

2. Bergey, D. H., and others: *Bergey's Manual of Determinative Bacteriology*, Baltimore, Williams & Wilkins Company, 1925.

3. Veillon, A., and Zuber: *Arch. de méd. expér. et d'anat. path.* 10:517, 1898.

4. Hallé, J., quoted by Teissier, Reilly, Rivalier and Stefanescu: *Ann. de méd.* 30:97, 1931.

which because of its extreme pleomorphism was called *Bacillus funduliformis*. Rist<sup>5</sup> and Guillemot, Hallé and Rist<sup>6</sup> isolated forms similar to those described by Veillon and Zuber and by Hallé from pulmonary abscesses, empyema and infected ears. Cottet<sup>7</sup> described these organisms as the cause of phlegmonous periurethral infections; Gilbert and Lippmann,<sup>8</sup> as one of the causes of cholecystitis, and Gohn and Sachs,<sup>9</sup> as an etiologic factor in peritonitis. The work of these pioneer investigators has been confirmed by many others, a review of whose studies appears in the article by Teissier, Reilly, Rivalier and Stefanescu.<sup>10</sup> Of equal importance are the observations of Tissier,<sup>11</sup> Distaso,<sup>12</sup> Debono<sup>13</sup> and Eggerth and Gagnon<sup>14</sup> that organisms of this group are found frequently, and even predominate, in the flora of the normal intestinal tract of man.

In 1931<sup>15</sup> and 1932,<sup>16</sup> Thompson and one of us (Beaver) reported two fatal cases of bacteremia due to gram-negative species of *Bacteroides*. The organism was identified in one case as *Bacteroides fragilis*, and in the other, as *Bacteroides funduliformis*. Infection was apparently primary in the genito-urinary tract of the male patient, with pulmonary abscesses secondary to the bacteremia. Although only two cases were reported, it was stated that similar organisms had previously been encountered in blood cultures in several cases, but that at the time of their appearance their significance was not recognized. Among these cases were two instances of carcinoma of the colon. A review of the literature during the period of these investigations revealed reports of ten instances of bacteremia due to gram-negative species of *Bacteroides*. In addition, a gram-positive species, *Bacteroides ramosus*, had been recovered in blood cultures.

Nonsporulating anaerobic bacilli of the genus *Bacteroides* in rare instances have been reported as the etiologic agents in hepatic abscesses.

5. Rist, Edouard: *Centralbl. f. Bakt.* **30**:287, 1901.

6. Guillemot, Louis; Hallé, Jean, and Rist, Edouard: *Arch. de méd. expér. et d'anat. path.* **16**:571 and 677, 1904.

7. Cottet, J., quoted by Teissier, Reilly, Rivalier and Stefanescu: *Ann. de méd.* **30**:97, 1931.

8. Gilbert, A., and Lippmann, A.: *Compt. rend. Soc. de biol.* **54**:1189, 1902.

9. Gohn, Anton, and Sachs, Milan: *Centralbl. f. Bakt.* **38**:131, 1906.

10. Teissier, P.; Reilly, J.; Rivalier, E., and Stefanescu, V.: *Ann. de méd.* **30**:97, 1931.

11. Tissier, H.: *Ann. de méd. et chir. inf.* **4**:675, 1900.

12. Distaso, Arcangelo: *Centralbl. f. Bakt.* **59**:97, 1911.

13. Debono, P.: *Centralbl. f. Bakt.* **62**:229, 1912.

14. Eggerth, A. H., and Gagnon, B. H.: *J. Bact.* **25**:389, 1933.

15. Thompson, Luther, and Beaver, D. C.: *Proc. Staff Meet., Mayo Clin.* **6**:372, 1931.

16. Thompson, Luther, and Beaver, D. C.: *M. Clin. North America* **15**:1611, 1932.

Norris,<sup>17</sup> in 1901, described a fatal illness due to multiple abscesses of the liver. There was an associated pylephlebitis, which suggests that the infection arose from within the distribution of the portal circulation. Two etiologic agents were described in this case: first, an anaerobic streptococcus similar to *Micrococcus fetidus* of Veillon, and second, an anaerobic bacillus similar to those which Veillon and Zuber had described as the causal factors in other suppurative processes. Harris,<sup>18</sup> in 1905, recorded a fatal illness characterized by multiple abscesses of the liver, lungs and spleen. The etiologic agent in this case was likewise recognized as similar to the anaerobic bacilli which had been described by Veillon and Zuber. Harris named the organism *Bacillus mortiferus*. Teissier, Reilly, Rivalier and Stefanescu, in 1931, reported four cases of bacteremia due to *Bacteroides funduliformis*. In one case the principal localization of the infection, probably the source of the bacteremia, was in the liver, where at necropsy multiple abscesses were found.

In a previous publication on hepatic abscesses by one of us (Dr. Beaver<sup>19</sup>) it was stated that staphylococci and streptococci were the usual etiologic factors. To these and other organisms enumerated should be added, apparently in a conspicuous position, members of the genus *Bacteroides*.

#### CLINICOPATHOLOGIC STUDY

We recently had the opportunity of studying two cases of abscesses of the liver in which *Bacteroides funduliformis* was identified as the etiologic agent. It seemed desirable to us that these cases be placed on record, since abscesses of the liver due to *Bacteroides funduliformis* have rarely been reported, and since they are expressions of infection with *Bacteroides* which are distinctly different from those previously reported from this institution.

In case 1, the primary lesion consisted of an infected, ulcerating carcinoma of the rectum, with metastasis to the regional lymph nodes, liver, lungs and left suprarenal gland. In the region of the rectal carcinoma, some veins were invaded by the growth and others were thrombosed. In some instances the thrombi were infected. Portal thrombosis and abscesses of the liver, closely associated with the metastatic carcinomas, were the terminal complicating factors. For obvious reasons it may be assumed that the hepatic abscesses and the hepatic metastatic growths of carcinoma arose through the same channel of dissemination, namely, from the rectum through the portal circulation.

17. Norris, Charles: J. M. Research **6**:97, 1901.

18. Harris, N. M.: J. Exper. Med. **6**:519, 1905.

19. Beaver, D. C.: Am. J. Path. **7**:259, 1931.



In case 2, no primary localization of the infection could be found at necropsy. The hepatic abscesses, however, had the general characteristics of abscesses disseminated by way of the portal circulation. They were large, somewhat multilocular and confined within the right lobe of the liver. It is probable, although not proved, that the hepatic abscesses in this case arose from a cryptic focus somewhere in the colon, appendix or rectum.

#### REPORT OF CASES

CASE 1.—A man, aged 64, for three months had had symptoms which were characteristic of rectal carcinoma. Proctoscopic examination and biopsy confirmed the diagnosis. A permanent left inguinal colonic stoma was made. Convalescence was uneventful until the seventeenth postoperative day, when there was a sharp rise in temperature to 102 F., with a pulse rate of 120 beats per minute. The fever continued as an irregular type, with morning remissions to a temperature of about 99 F. and afternoon rises to 100.2 or 102 F., and there were occasional chills. The patient became jaundiced; the bilirubin content was from 7.9 to 11.5 mg. per hundred cubic centimeters of serum, and the van den Bergh reaction was direct. The number of leukocytes ranged from 19,600 to 25,900 per cubic millimeter of blood. The course was characterized by persistent, irregular fever and gradual decline, and the patient died three weeks after the fever began.

At necropsy the recent right rectus incision made for colostomy was well healed and in good condition. The peritoneal cavity contained 2,000 cc. of clear, light yellow fluid. The rectum revealed a large, annular polypoid, ulcerated and infected carcinoma. Metastasis had occurred to the regional fascia and lymph nodes and also to the aortic lymph nodes and the liver, lungs and left suprarenal gland. The liver weighed 3,670 Gm. It was greenish brown. The surface was nodular, the nodules having an appearance typical of metastatic carcinoma. The central portions of the nodules were usually firm and umbilicated, but in some nodules these areas were elevated and soft. Nodules were abundantly present throughout all parts of the liver. Those with softened central parts were revealed as metastatic carcinomas with central suppuration. The pus had a mildly disagreeable, rancid odor and was yellow. The extrahepatic and intrahepatic portions of the portal vein were thrombosed. In neither the wall of the vein nor the thrombus was there evidence of suppurative inflammation. Other organs were essentially normal.

Microscopically, the rectal neoplasm was a malignant epithelial cell growth, composed of newly formed, atypical glandular acini in polypoid formation. The surface of the growth was ulcerated and covered by a pseudomembrane, consisting of polymorphonuclear leukocytes, serum, fibrin and necrotic cellular detritus. The growth had extended into the muscularis, the surrounding fatty areolar tissue and some of the veins. In other veins there were thrombi in which were seen areas of necrosis with dense collections of polymorphonuclear leukocytes. The liver exhibited rather intense sinusoid congestion, frequently with central atrophy and necrosis. Sections of the nodular portions disclosed a malignant epithelial neoplasm, similar in appearance to the rectal carcinoma. The walls of the abscesses were composed of an outer zone of metastatic carcinoma and an inner zone of fibrous connective and granulation tissue; the more peripherally situated tissue consisted of rather dense, mature fibrils, whereas the inner portion was composed mostly of fibroblasts and young blood vessels. Throughout the walls were present

also polymorphonuclear neutrophilic leukocytes, lymphocytes and large mononuclear phagocytic cells. Within, the neutrophilic leukocytes gradually became predominant until the central zone of suppuration was approached. Here only polymorphonuclear neutrophils, fibrin, coagulated serum and necrotic detritus were found. More centrally the exudate was almost entirely necrotic. The abscesses were formed either entirely within a metastatic tumor nodule or at its side. Sections of the portal vein revealed a recently formed, bland thrombus, the peripheral portions of which were being organized. Metastatic carcinomatous nodules having a structure similar to that of the nodules in the rectum and liver were also found in the lymph nodes, lungs and left suprarenal gland. None of these nodules was infected.

CASE 2.—A man, aged 50, about Jan. 27, 1933, contracted what he thought was influenza. On February 9, he was seen by physicians of the Mayo Clinic. He complained of having vague pains in the right lower part of the thorax anteriorly and in the right upper quadrant of the abdomen, with cough, fever and weakness. On February 7, he had had a chill. Physical examination revealed him to be well developed, poorly nourished and dehydrated. He was breathing rapidly and was somewhat dyspneic. Examination of the thorax disclosed no definite limitation of movement on either side and no definite impairment of resonance, but the breath sounds seemed to be suppressed over the bases of both lungs, and a few crackling râles were heard over the base of the right lung. The abdomen was held rigid, and some tenderness was elicited on palpation below the right costal margin. The liver was not palpable. The temperature was 102 F.; the pulse rate, 96 beats per minute, and the blood pressure, 130 mm. of mercury systolic and 100 mm. diastolic. Roentgenologic examination of the thorax revealed bilateral elevation of the diaphragm and infiltration of the right cardiophrenic angle. The concentration of hemoglobin was 13.3 Gm. per hundred cubic centimeters of blood, and the leukocytes numbered 14,300 per cubic millimeter of blood, 90 per cent being polymorphonuclear neutrophils, 6 per cent, lymphocytes, and 4 per cent, monocytes. The fever became distinctly septic and was associated with severe chills and sweats. There were extreme variations between morning and afternoon temperatures, as recorded in figure 1. Leukocytosis persisted, the white cell count varying between 13,900 and 55,300 per cubic millimeter of blood; usually the count was more than 25,000, with about 90 per cent polymorphonuclear neutrophils in the differential smear. In two blood cultures there was no growth of micro-organisms. Mild icterus developed a few days before death. The course was characterized by progressive weakness, with gradual decline, and death occurred on March 7, 1933, forty days after the illness began.

At necropsy, several small, sharply demarcated nodular regions were found in the lower lobes of both lungs; these had a grayish-white, caseous center and a hemorrhagic periphery. The central portion of some of these had undergone suppuration, forming abscesses from 1 to 1.5 cm. in diameter. The liver weighed 3,190 Gm. The capsular surface was brownish red and generally smooth and glistening. Some portions rose above the general contour of the surface as nodular areas of irregular size which fluctuated on palpation. Throughout the right lobe there were multiple multilocular and unilocular abscesses. The pus had a mildly putrid, somewhat acrid odor and was thick and greenish yellow. In the periphery of the abscesses it was often more caseous than purulent. The abscesses varied in diameter from 0.5 to 10 cm., the smaller ones being arranged in clusters about the walls of a larger one, thus giving the area around the large abscess a multilocular appearance whereas the central abscess itself was almost entirely unilocular. There were no other significant pathologic changes.

Microscopically, in the lower lobes of the lungs there were small focal zones of bronchopneumonia. In other parts there were small abscesses from the central portions of which alveolar structures had disappeared and had been replaced by dense collections of polymorphonuclear neutrophilic leukocytes and also by zones of necrosis. In the exudate from the peripheral zone especially there were masses of bacteria which stained faintly with hematoxylin. In some sections there were only focal areas of hemorrhage. In the hepatic abscesses the exudate was composed centrally of granular, faintly eosin-stained, almost acellular material, but nearer the periphery it appeared as definite collections of fairly well preserved polymorphonuclear neutrophilic leukocytes, though areas of necrosis were still present. In this zone there were also many masses which stained faintly with hematoxylin—apparently colonies of micro-organisms. A few fibroblasts, newly formed capillaries, lymphocytes and large mononuclear phagocytes were also associated with this zone. In the abscess wall proper, connective tissue gradually became more dense, being at first predominatingly fibroblastic with a few newly

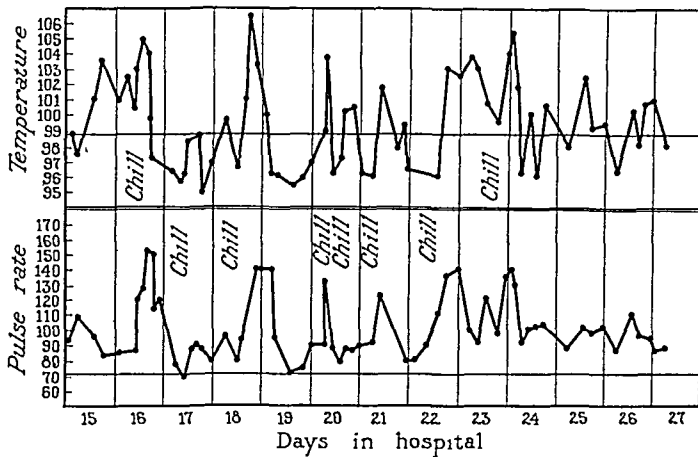


Fig. 1 (case 2).—A typical portion of the record of the temperature and the pulse rate from the twenty-eighth to the fortieth day of illness. Death occurred on the twenty-seventh day after admission and the fortieth day after the illness began.

formed capillaries and being transformed gradually into a peripheral encircling zone composed of mature fibrous connective tissue. Intermingled with the fixed tissue of the abscess wall there were a few polymorphonuclear neutrophilic leukocytes and a greater number of lymphocytes and large mononuclear phagocytes.

#### CLINICAL STUDY

On the basis of a review of cases reported in the literature and of personal experience, infections due to *Bacteroides funduliformis* and other species impress us as being worthy of serious consideration. This is true not only because infections of this type seem not to be rare but because they are serious and apparently often, if not usually, fatal.

So far as clinical manifestations of the pathogenicity of *Bacteroides* are concerned, there are no definite differential diagnostic points which

set this type of infection off from other severe pyogenic reactions. Blood cultures frequently become positive during the course of illness, but not always early; they should therefore be repeated if bacteremia is suspected. When positive, they serve as a means of identifying the type of infection. The symptoms and physical signs which are evoked naturally depend primarily on the points of localization. If there are distinctive features, however, they may be found in extreme toxic manifestations, as expressed by a high, septic type of fever, associated with chills, perspiration and a profound state of weakness progressing to exhaustion. These signs may be extreme and disproportionate to the actual physical expression of infection. The primary as well as the secondary points of localization may remain more or less obscure.

With the occurrence of infection of the liver, there were the usual clinical evidences of hepatic suppuration, which were expressed not only by the regional physical findings but by the high, irregular type of fever, with marked morning remissions and high afternoon exacerbations. There were associated chills and perspiration, with systemic evidence of severe toxicity and terminal jaundice. There was also marked leukocytosis, with polymorphonuclears predominating.

The pulmonary complications which accompanied one of the cases reported here were similar to those which Thompson and Beaver previously described. They consisted of septic infarction, bronchopneumonia and abscesses. Guillemot, Hallé and Rist and recently Cohen<sup>20</sup> have emphasized the pulmonic manifestations of infection with *Bacteroides*, particularly stressing putrid or gangrenous pulmonary abscesses and empyema. Pulmonary lesions may be only terminal embolic phenomena, appearing after an already established infection has provoked severe reaction. The emboli may invade the lung from primary and secondary situations elsewhere, as they did in case 2, or they may occur early in the course of illness, representing the secondary embolic localization of the infection from a primary situation elsewhere, as they did in the cases described by Thompson and Beaver. In the experience of others, pulmonary infections may be primary, apparently the result of aspiration, and may lead to putrid and gangrenous pneumonia, abscesses and empyema.

#### PATHOLOGIC STUDY

In our studies the primary lesions in infections with *Bacteroides* were found either in the genito-urinary tract, as previously reported by Thompson and Beaver, or in the colon, as in the cases reported here. They have been described by others as occurring in other portions of the body, as enumerated at the beginning of this paper. The colonic lesion may occur at the site of ulcerating carcinoma, as in case 1, or from

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20. Cohen, John: Arch. Surg. 24:171, 1932.

some unrecognized focus, as in case 2. In the latter instance the assumption that a lesion existed in the colon is only presumptive, although there is some support for this conclusion. If the primary lesion is colonic, it is within the distribution of the portal circulation, and secondary manifestations of embolic hematogenous dissemination will be found in the liver, as they were in the cases reported here. If the primary lesion is low in the rectum, the direct dissemination of the infection may be through the caval circulation, with secondary embolic manifestations in the lungs. This was apparently also the route of dissemination of the infection from primary localizations in the prostate gland and urinary bladder, as previously referred to in the cases reported by Thompson and Beaver. If secondary infection results in the liver, the lungs and other tissues eventually may become involved as the bacteremia becomes established in the pulmonary and general circulation, as in our case 2; likewise, if the secondary localization is pulmonic, other organs may become involved as the bacteremia invades the general circulation. In the cases reported by other investigators the infection has sometimes had a more widespread dissemination than we have seen, involving the lungs, pleura, liver, kidneys and skin.

*Bacteroides funduliformis* and other pathogenic species are probably normal inhabitants of the oral and pharyngeal cavities, and most assuredly of the intestinal tract, as similar species have been found there by other investigators. Just how and why these organisms become pathogenic is unknown, but it may be assumed that while in their natural saprophytic existence they ordinarily cannot evoke inflammatory reactions, as soon as local injury occurs or they become transported to tissues elsewhere which by nature do not favor the saprophytic existence of bacteria they become distinctly parasitic and provoke a profound toxic reaction. Teissier and his co-workers felt that the primary localization of this type of infection may be pharyngeal. This might account for the frequency with which the organisms have been found in pulmonary abscesses and empyema. Infection of the intestinal tract and structures in close anatomic relationship seems to make it evident that the primary focus may be in these structures. This opinion is supported by the finding of organisms of this genus as one of the dominant types of organisms in the intestinal flora. It receives further support from the occurrence of infection of certain portions of the intestinal tract and of the male and the female genital tract and from the finding of abscesses of the liver caused by *Bacteroides* coexistent with metastatic colonic carcinoma, as in our case 1.

The pathologic anatomy of infection with *Bacteroides* as revealed in the liver and lungs in our cases was somewhat distinctive. The abscesses in the liver were large, with a tendency to be multilocular, especially in

the peripheral portion (fig. 2 *A*). The exudate was thick, tenacious and usually distinctly purulent, although in smaller lesions it tended to be caseous. The exudate was from white to light yellow or greenish yellow, and it emitted a peculiar, mildly putrid or acrid odor sometimes resembling the odor of butyric acid. The abscesses were usually well encapsulated by fibrous connective tissue, in which newly developed vascular channels formed a prominent part. Most of them were chronic (fig. 2 *B*). In the exudate, polymorphonuclear neutrophilic leukocytes

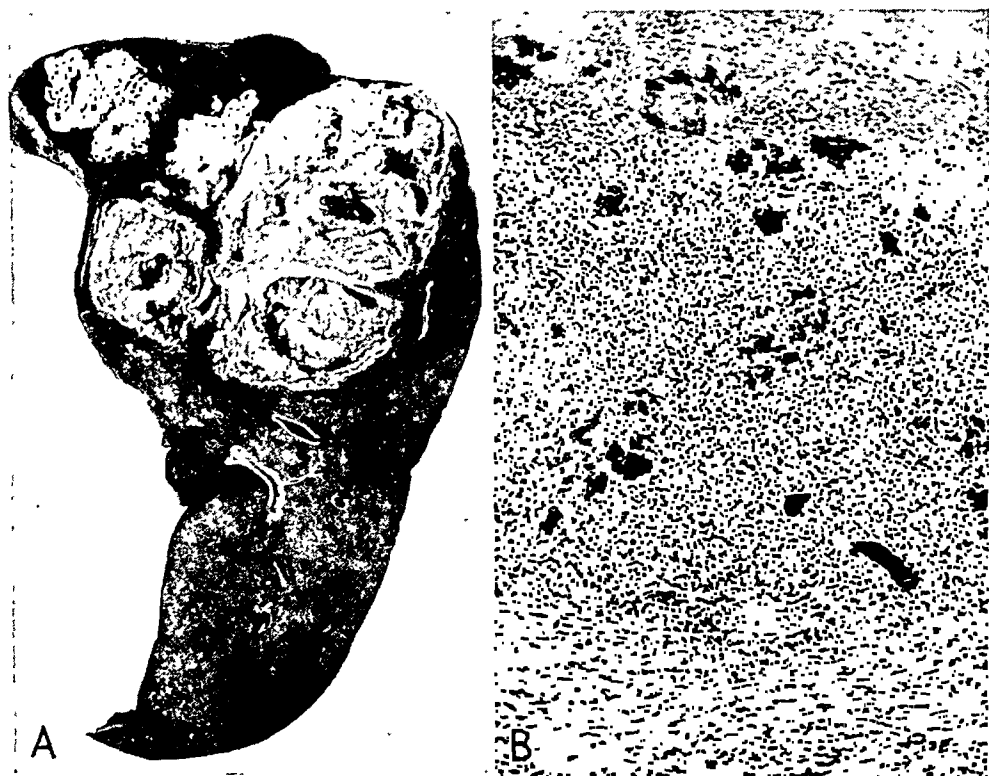


Fig. 2 (case 2).—*A* shows large multilocular and unilocular abscesses in the right lobe of the liver. *B*, a microscopic view of an abscess. Numerous colonies of micro-organisms are prominent (hematoxylin and eosin;  $\times 100$ ).

predominated. The fixed tissues in the region of the infection, as well as the exudate itself, were partially necrotic. In the lungs the lesions had the appearance of small, septic or bland infarcts, abscesses or patches of bronchopneumonia. Usually the lesions were solid, firm and grayish or of a hemorrhagic color. The grayish nodules appeared as solid regions of necrosis, somewhat caseous. All transitional forms existed to small abscesses with definite suppuration. Large, putrid or gangrenous abscesses in the lungs, with putrid empyema, such as have been reported by others, were not observed. Such lesions as these are apparently primary in the lung and usually the result of aspiration.

Primary lesions of the genito-urinary tract and colon, as we have observed them, are less distinctive, since the bacterial flora in these situations has always been mixed.

Abscesses of the liver caused by *Bacteroides funduliformis* must be distinguished from amebic abscesses, actinomycotic abscesses and abscesses due to other pyogenic micro-organisms, such as staphylococci and streptococci. Grossly, they all bear a superficial resemblance to one another, although there are certain features which may serve to distinguish them. Amebic abscesses are usually solitary and unilocular and associated with amebic colitis, although the lesions in the colon may be healed. Actinomycotic abscesses, as a rule, have heavy fibrous and granulomatous tissue capsules, frequently with associated sinus tracts. Abscesses of the staphylococcic or the streptococcic type more often are associated with suppurative pylephlebitis and have a more distinct multilocular or honeycomb appearance, similar to that of the abscesses caused by *Actinomyces*. The final differential diagnosis is dependent on microscopic and bacteriologic examinations. Of course, amebic colitis may be preceded or followed by infection of the colon with pathogenic bacteria or vice versa. This possibility must be considered in connection with organisms of the genus *Bacteroides*. However, in the cases of abscesses of the liver caused by *Bacteroides* which we have studied, no evidence of primary or secondary amebic infection has been found.

#### BACTERIOLOGIC STUDY

The bacteriology of *Bacteroides* is the particular obstacle which has led to the relative lack of recognition of this type of infection for so long. The organisms are obligate anaerobes, and the pathogenic varieties at least develop on culture mediums with much difficulty except when the medium is enriched by blood, serum or tissues. When mixed infection exists, their isolation may prove still more trying. However, when they are obtained in blood cultures, the culture is usually pure. Their biologic reactions are extremely difficult to observe because they do not grow readily on differential mediums. They are extremely pleomorphic, often being filamentous.

The cultural characteristics of *Bacteroides funduliformis* recently (1931) were well studied by Teissier, Reilly, Rivalier and Stefanescu with organisms isolated from four cases of this infection. The organisms in our cases agree in all essentials of morphologic structure and biologic reactions with those described by the investigators named. Since in our cases the two strains were practically identical morphologically and culturally, they will be described together, exceptions being noted whenever necessary. In case 1 the organism was isolated from the hepatic abscess only, but in case 2 the cultures were obtained from both the hepatic abscess and the blood of the heart.

Microscopic sections of fixed tissue from the hepatic abscesses were stained by Brown's modification of Gram's stain. In case 1, the organisms appeared in small clusters or tufts in the peripheral portion of the exudate. They were irregularly placed in a sort of criss-cross arrangement. The individual organisms appeared as plump, straight, long, unbranched rods and filaments with rounded ends. They were gram-negative (fig. 3 *A*). In case 2, the organisms were relatively short,

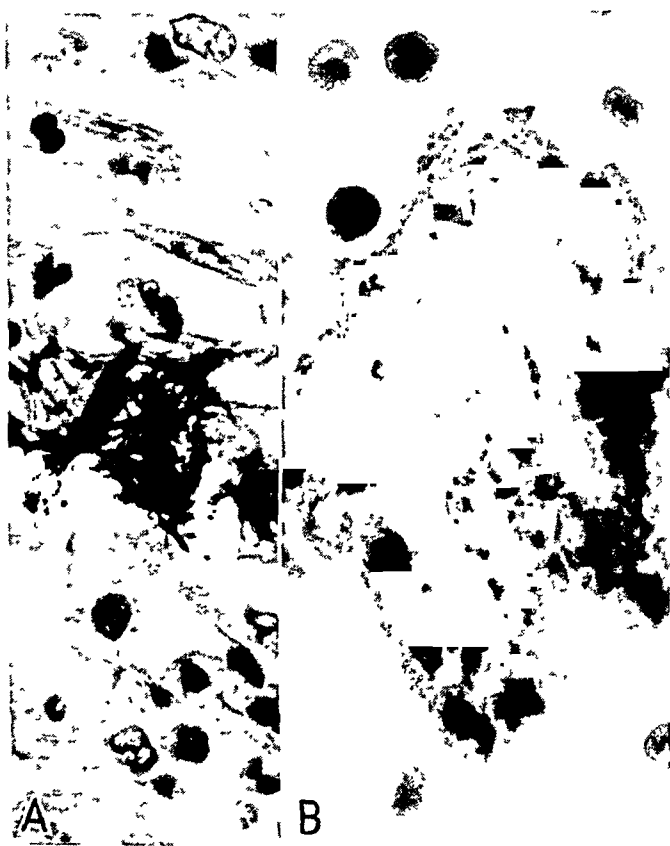


Fig. 3.—*A* shows a colony of micro-organisms in a section of tissue from the hepatic abscess in case 1 (Brown-Gram stain;  $\times 1,700$ ). *B* shows a colony of micro-organisms in a section of tissue from the hepatic abscess in case 2 (Brown-Gram stain;  $\times 900$ ).

plump, gram-negative bacilli with rounded ends, frequently with bipolar granules, and were usually closely massed in clusters, making the identification of individual forms extremely difficult (fig. 3 *B*). In this case similar masses of organisms were found also in the pulmonary abscesses.

On culture mediums marked morphologic variations were observed, apparently dependent on the age of the culture, the type of medium employed or other unknown factors of growth. In a general way, the



more extreme variations in involution were found in cultures on solid mediums, such as hormone blood agar, hormone serum agar and Löffler's serum, although varying degrees of dissociation were observed in cultures in liquid mediums, especially in certain of the dextrose broth solutions. In dextrose-brain broth the usual form was a short, plump rod with rounded ends, or a slender, slightly longer, rod, likewise with rounded ends. Occasionally filaments were seen. The rods frequently had deeply stained granules or bars distributed throughout the



Fig. 4.—*A* shows coccobacillary and filamentous forms in a forty-eight hour brain broth culture (carbolfuchsin stain;  $\times 1,500$ ). *B* shows comma-shaped forms in a twenty-four hour brain broth culture from the peritoneum of rabbit 1 (carbolfuchsin stain;  $\times 1,250$ ).

cell, only polar granules being present in the shorter forms. The latter appeared as coccobacillary forms, which when in chains closely resembled chains of streptococci (fig. 4 *A*). Occasionally the rods were evenly stained, and in these, especially when filamentous, dissociation into granular or streptococcic types could be observed in all gradations from uniformly staining cells to chains of granules resembling streptococci. Slender, comma-shaped rods were further variants (fig. 4 *B*). Clumping of the organisms was usual. In older broth

cultures there was a gradual reduction in the number of stainable organisms; preparations of old but living cultures had the appearance of amorphous detritus. Young cultures on solid mediums were similar to young cultures on brain broth, but as the cultures on solid mediums increased in age, large numbers of swollen forms appeared (fig. 5 *A*). These were also seen to some degree in older broth cultures, but were less numerous. The swollen forms were often cigar-shaped, with bluntly rounded ends; the cell bodies were faintly stained, and deeply

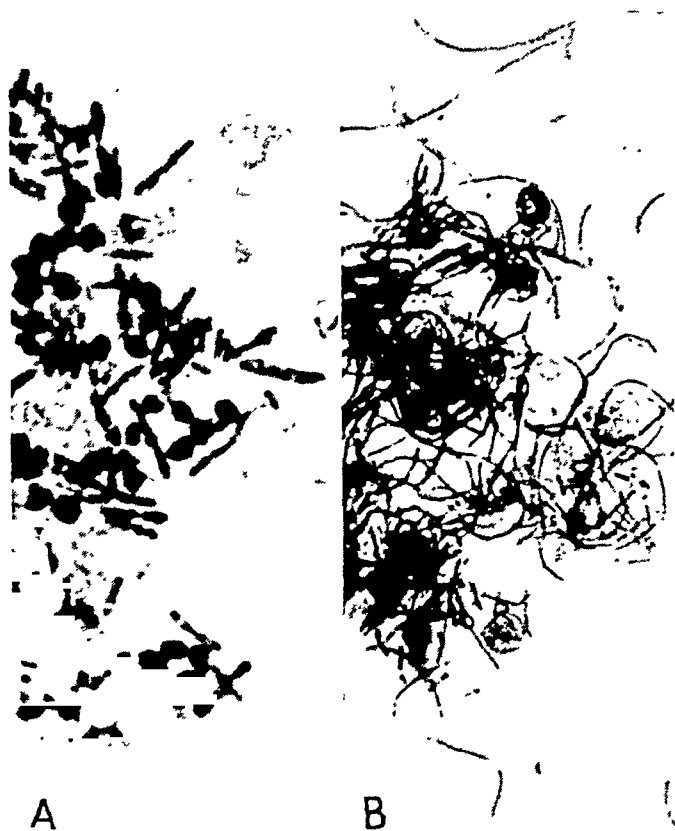


Fig. 5.—*A* shows swollen, typical types of *Bacteroides funduliformis* in a four day brain broth culture (carbolfuchsin stain;  $\times 1,800$ ). *B* shows filamentous forms in a five day blood agar culture (carbolfuchsin stain;  $\times 825$ ).

stained granules, beads or bars were present. The swollen forms were at other times oval or spherical. The oval or spherical forms were frequently deeply stained, without granules; some, however, were almost invisible, so faintly did they take the dye. On solid mediums, unbranched, uniformly stained filaments frequently predominated. These often were arranged in tufts (fig. 5 *B*). Less frequently the filaments were faintly stained, only the outer envelop becoming visible; this envelop surrounded an unstained, tubelike or hypha-like structure in which there were small stained granules or bars. These filaments

revealed, here and there, oval enlargements, which appeared to be the result of longitudinal splitting and swelling of the filaments, rather than spores, which they resembled. On further study such forms always reverted to the original coccobacillary type; otherwise one might assume that they represented contaminants.

The organisms were always gram-negative and never acid-fast. They were nonmotile. The usual aniline dyes, such as safranin and methylthionine chloride, U. S. P. (methylene blue), stained them faintly. They were well stained by dilute carbolfuchsin.

Cultures in dextrose-brain broth were allowed to stand at room temperature. After seven months (case 1) and four and a half months (case 2) they were still viable. They were readily susceptible to heat; a temperature of 56 C. was sufficient to kill cultures on dextrose-brain broth in two and five minutes (cases 1 and 2 respectively).

Dextrose-brain broth was found to be the most satisfactory medium for routine culturing of the organisms. Under the conditions present in this medium no precautions were necessary for obtaining growth anaerobically. Visible growth appeared in from one to seven days at 37.5 C. The rapidity of the appearance of growth apparently depended on the degree of anaerobiosis and on the number of organisms present in the inoculum. Evidence that growth was present became manifest at first by the formation of bubbles of gas in the region of the particles of brain at the bottom of the tube. As the bubbles enlarged they tended to rise to the surface of the tube. Rarely was gas formed so rapidly that bubbles accumulated on the surface of the medium. Next to the formation of gas, the medium gradually became turbid in the region of the particles of brain, this progressing to form eventually a layer of fine flocculi overlying the brain. Usually, unless disturbed, the medium remained clear, but occasionally it became slightly cloudy. The cultures had a mild, stale, putrid odor which gradually took on the odor of butyric acid. The brain was not blackened or digested. Tests of the gas rising from the tubes were negative for hydrogen sulphide.

No growth appeared on hormone agar, hormone blood agar, hormone serum agar or plain broth when allowed to incubate aerobically at 37.5 C. for several days. Satisfactory conditions for growth were obtained in these and other mediums by the use of pyrogallol and sodium hydroxide to absorb the oxygen. Anaerobic plating was successful in Spray plates. Slanted solidified mediums—gelatin, broth, milk, sugar fermentation tubes and similar preparations—were inoculated and placed in a Novy type anaerobic jar, with pyrogallol and sodium hydroxide added as before mentioned.

On hormone blood agar Spray plates, after twenty-four hours' incubation at 37.5 C., very small, scarcely visible, pinpoint-like colonies

appeared. A small zone of partial hemolysis surrounded each colony on examination with a hand lens. After forty-eight hours' incubation, surface colonies were discrete, slightly translucent, round, flat or slightly convex, moist, bluish gray and 1 mm. or less in diameter. A hemolytic zone surrounding each colony became distinct. Deep colonies were grayish white, opaque, round or lens-shaped and 0.5 mm. or less in diameter. A clear zone of hemolysis, from 1 to 3 mm. in diameter, surrounded each deep colony. At this time the growth resembled in appearance that of hemolytic streptococci. As plates were observed over a period of days, surface colonies became flat and rather dry and frequently had a deep opaque center, with a surrounding halo-like ring of growth. Deep, lens-shaped colonies and others became irregular, often with formations resembling Maltese crosses.

On hormone human blood agar slants, after forty-eight hours there was abundant growth of small, discrete, rounded, moist, somewhat translucent and slightly convex, bluish-gray colonies along the line of inoculation. Hemolysis was present. The condensation water became cloudy. On hormone human serum agar slants, growth was similar but less abundant. On plain hormone agar slants, usually there was no visible growth after two weeks; an occasional colony similar to those on blood agar appeared. On plain agar slants there was no visible growth after two weeks. On plain gelatin, there was no visible growth or liquefaction after two weeks. On gelatin plus human blood serum, sparse, small, irregular-shaped colonies appeared; there was no liquefaction at the end of two weeks.

In plain broth there was no visible growth after two weeks. In plain broth with coagulated egg white added, the medium remained clear and there was a slight flocculent growth at the bottom of the tube. There was no digestion of egg at the end of two weeks. On Löffler's blood serum there were almost imperceptible fine, discrete, slightly elevated colonies. There was no liquefaction of the serum after two weeks. There was no change in litmus milk after two weeks.

Peptone fermentation medium, with various sugars added, was inoculated and incubated anaerobically at 37.5 C. Growth failed to appear. Similar tubes to which 0.5 cc. of fresh human serum was added revealed abundant growth. Acid and gas were formed in dextrose, levulose and maltose. There was neither acid nor gas in sucrose, mannitol, xylose, galactose or lactose. Other sugars were not tried.

#### EXPERIMENTAL STUDY

Tests for pathogenicity were made on four guinea-pigs and nine rabbits. Guinea-pigs 1 and 2 and rabbits 4, 5, 6 and 7 were inoculated with forty-eight hour dextrose-brain broth cultures of blood from

case 1. Rabbit 1 was inoculated intravenously with 0.5 cc. of a dilution in physiologic solution of sodium chloride of pus from an abscess of the liver in case 2. The remaining two guinea-pigs (3 and 4) and four rabbits (2, 3, 8 and 9) were inoculated with forty-eight hour dextrose-brain broth cultures of blood from case 2. Inoculations were carried out in guinea-pigs subcutaneously and intraperitoneally and in rabbits subcutaneously and intravenously. In general, the organisms

*Results of Animal Inoculations*

Animal	Dose* and Method of Administration	Time Between Injection and Death	Result	Organism Recovered From
Guinea-pig 1	2.5 cc. subcutaneously	14 days (killed)	Subcutaneous abscess; drained spontaneously	Abscess
Guinea-pig 2	2 cc. intraperitoneally	21 days (killed)	No pathologic change	
Guinea-pig 3	1.5 cc. subcutaneously	33 days (killed)	Some early induration; recovered	
Guinea-pig 4	2 cc. subcutaneously	16 days (killed)	Subcutaneous abscess; drained spontaneously	Abscess
Rabbit 1	0.5 cc. intravenously (pus from abscess of liver)	Less than 15 hours	Beginning peritonitis	Peritoneum
Rabbit 2	0.6 cc. intravenously	5 days	Death due to other causes	
Rabbit 3	2 cc. subcutaneously	11 days (killed)	Subcutaneous abscess	Abscess
Rabbit 4	0.6 cc. intravenously	3 days	No pathologic change	
Rabbit 5	2 cc. subcutaneously	11 days	Subcutaneous abscess; multiple small hepatic abscesses, up to 1 mm. in diameter	Subcutaneous abscess; liver
Rabbit 6	0.5 cc. intravenously	51 days (killed)	Moribund on fourth day; recovered	
Rabbit 7	1.5 cc. intravenously	8 days	No pathologic change	
Rabbit 8	1.5 cc. intravenously	9 days	Purulent pleurisy; two hepatic abscesses, 1 cm. in diameter	Blood; hepatic abscess
Rabbit 9	0.5 cc. intravenously	14 days	Purulent pleurisy	Blood; pleural pus

\* All injections, except for rabbit 1, were of forty-eight hour brain broth cultures.

were found to be somewhat less virulent than Thompson and Beaver had previously reported. The reactions, however, were similar. The results are summarized in the table.

Macroscopically, all of the abscesses contained thick yellow pus, which emitted a mildly putrid odor. Microscopically, they were usually chronic, with well defined granulomatous reactions. They contained a central area of necrotic material in which only a few leukocytes were found. Polymorphonuclear neutrophils and large mononuclear leukocytes became predominant peripherally. Mononuclear forms and lymphocytes gradually increased in number in the wall of the abscess, where fibroblasts and newly formed blood vessels also were present. Rarely giant cells were observed in this granulomatous zone. Sur-

rounding each abscess, connective tissue finally became dense and fibrous. Some of the abscesses were multilocular. In the muscle bundles near the subcutaneous abscesses there were granular degeneration and fragmentation, and about the vessels there were perivascular collections of leukocytes and lymphocytes. Gram stains of sections demonstrated that the bacteria occurred in dense masses of short, plump, coccobacillary forms within the abscess cavity. In a section of the subcutaneous abscess of rabbit 3, the organisms appeared in clusters of filamentous forms, as they did in sections of a hepatic abscess in case 1. The organisms in all sections studied were gram-negative.

#### SUMMARY

Two cases of hepatic abscess caused by *Bacteroides funduliformis* have been presented. The genus *Bacteroides* (Bergey) comprises certain anaerobic, nonsporulating bacilli. Organisms of this type were first described by Veillon and Zuber and by Hallé as the etiologic agents in various suppurative and gangrenous infections of man. Similar species also have been isolated from the normal intestinal flora of man.

It is probable that infections of this type are not rare and that there are two regions which seem particularly vulnerable to primary infection, namely, the large bowel and the genito-urinary tract of the male. In the colon, infection appears to take origin principally in infected carcinomas, although the focus may be cryptic. From the primary focus, direct hematogenous dissemination may occur to the liver or the lungs, with clinically demonstrable bacteremia frequently supervening. Such infections are extremely serious and entail a grave prognosis. The clinical syndrome produced is an extreme degree of toxicity, with a high, remittent type of fever, chills, perspiration and weakness which progresses to exhaustion. The physical findings vary with the points of localization.

The lesions of the liver, as we have observed them, have been essentially chronic granulomatous abscesses, frequently multilocular and spreading with extreme destructive changes, necrosis of tissue and exudation. In the lungs, the lesions have appeared as small septic infarcts, patches of bronchopneumonia or small abscesses. Most commonly found were firm, solid, grayish or hemorrhagic nodules, the grayish areas resembling beginning caseation necrosis. Other investigators have described large putrid or gangrenous abscesses of the lungs with empyema associated with organisms of this group. Such lesions are apparently primarily pulmonic, usually on the basis of aspirated infection. Guinea-pigs and rabbits are susceptible to experimental inoculation with *Bacteroides funduliformis*. The lesions produced are abscesses, similar in appearance to those occurring in man.

# ISOLATION OF ETHER FROM HUMAN TISSUES

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The problem of proving definitely the presence of ethyl ether,  $(C_2H_5)_2O$ , in human tissues has remained unsolved to the present time. There are no specific color or precipitation reactions for ether. Methods used by Nicloux,<sup>1</sup> Shaffer and Ronzoni,<sup>2</sup> Haggard<sup>3</sup> and Spenzer<sup>4</sup> for the quantitative determination of ether are based on oxidation-reduction reactions. These are not specific qualitative tests for ether. Many organic substances, such as hydrocarbons, alcohols, aldehydes, ketones and organic acids, react as ether does toward these oxidizing agents. In medicolegal cases the pathologist or toxicologist has to rely on his sense of smell. When the body cavities are opened, he seeks the odor of ether. Proof of this kind is unsatisfactory and often misleading. For example, odors of various kinds may be encountered in body cavities and in organs which at times may simulate that of ether and at other times may conceal the odor of ether when it is present. The problem we set out to solve was the actual isolation of ether in pure form from the organs and its identification by determination of the boiling point.

Gettler, Niederl and Benedetti-Pichler,<sup>5</sup> in their contribution on the isolation of ethyl alcohol from human tissues, devised a specially constructed rectification flask. This apparatus was used at first in the attempt to isolate ether from tissue distillates. It served the purpose quite well. We succeeded in isolating droplets of ether, proving the same by determinations of the boiling point.

It was found that certain structural changes in the rectification flask not only would facilitate the isolation of the ether, but would also allow

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Contribution from the chemical laboratories of the Chief Medical Examiner's Office, Bellevue Hospital, and of Washington Square College, New York University.

1. Nicloux, M.: *Compt. rend. Soc. de biol.* **61**:606, 1907.
2. Shaffer, P. A., and Ronzoni, E.: *J. Biol. Chem.* **57**:741, 1923.
3. Haggard, H. W.: *J. Biol. Chem.* **56**:127, 1923.
4. Spenzer, J. G.: *Arch. exper. Path. u. Pharmacol.* **33**:407, 1893.
5. Gettler; Niederl and Benedetti-Pichler: *Mikrochemie* **11**:173, 1932; *J. Am. Chem. Soc.* **54**:1479, 1932.

the collection and measurement of practically all of the ether present in the tissues. The changes in the microrectification flask (fig. 1) are:

1. The bulb of the flask is of larger capacity (300 cc.). This enables one to use the entire original tissue distillate rather than small aliquot portions. In this way the volumes of ether isolated are large enough to be measured.

2. A glass tube running to the bottom of the flask is fused into the side of the flask. This facilitates the introduction of the material from

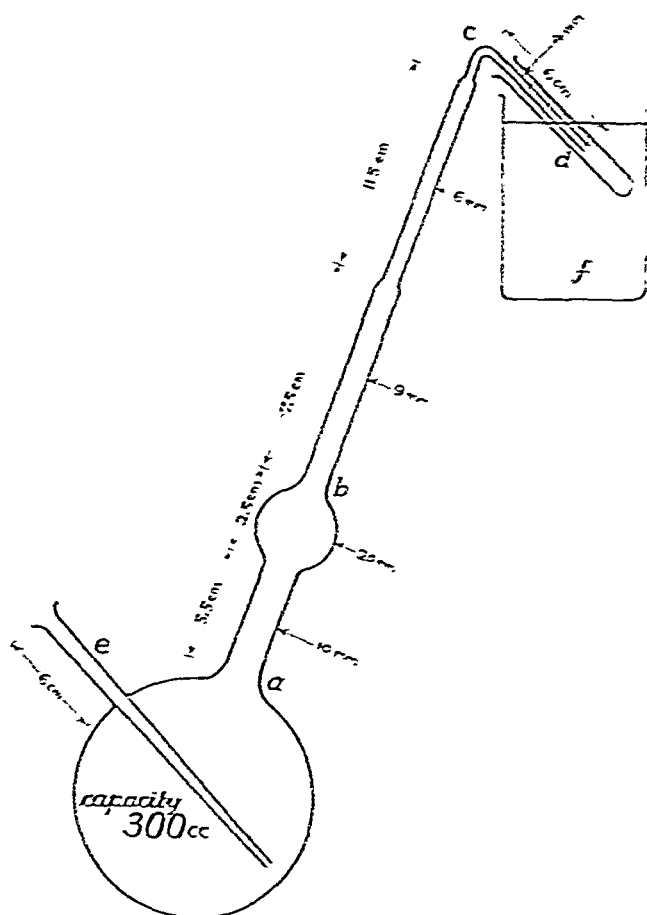


Fig. 1.—Microrectification flask.

which the ether is to be isolated, serves as a safety tube during the distillation and makes it easier to empty and clean the flask.

3. The uppermost S-shaped part is straightened out and points downward at an angle of 45 degrees to the main tube of the flask. This was done so that all the ether could be collected in one portion rather than in a series of successive drops.

#### METHOD OF ISOLATING ETHER FROM TISSUES

The tissues or organs removed at autopsy are placed quickly in glass jars, tightly sealed and put in a refrigerator. When ice cold, about 60 Gm. is quickly



ground up. The grinder is previously cooled by applying ice. Of this ground material, 500 Gm., is rapidly weighed out, mixed with 200 cc. of ice cold water and placed in a 2 liter flask. An additional 300 cc. of water and 1 cc. of liquid petrolatum are added. The mixture is distilled with steam, a long, well cooled condenser, the tip of which has been bent to resemble an adapter, being used. The bent tip should dip into 25 cc. of ice water contained in the receiving flask, the latter being at all times entirely surrounded by ice. Two hundred cubic centimeters of distillate is collected. This is ample for recovering all the ether that is present in the tissues. The distillate is now ready for the isolation of the ether.

The rectification flask already described is used. It must be clean, and part of it (from *b* to *d*) must be dried by playing a gentle flame over the outside surface.

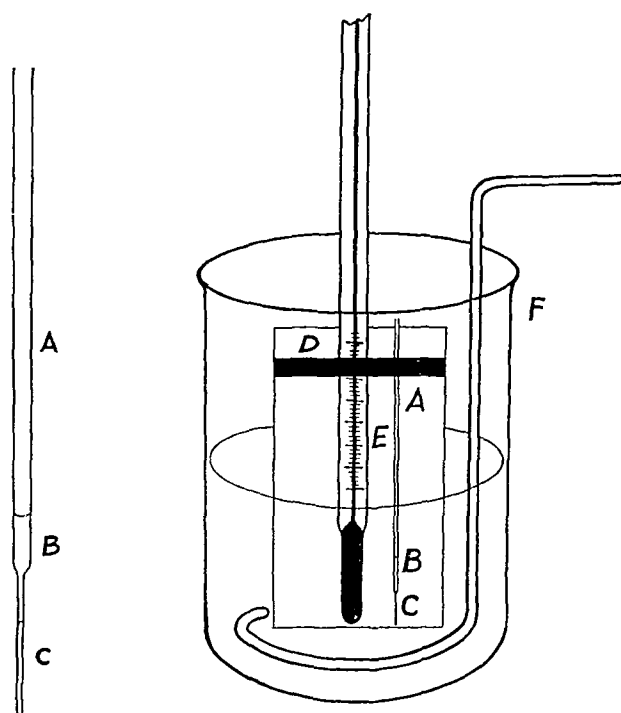


Fig. 2.—Microboiling point apparatus.

The entire 200 cc. of distillate is poured through the safety tube (*c*) into the rectification flask. One gram of granulated zinc is added. The receiving tube (*d*), which is 6 mm. in diameter on the inside and is graduated in twentieths of a cubic centimeter, is surrounded by a bath (*f*) of solid carbon dioxide in acetone, and should contain about 0.15 cc. of water (not more), so that the ether is collected over water as a separate layer. The rectification flask is now heated carefully and slowly, an asbestos centered wire gauze and a microburner being used. As soon as the solution begins to boil, the flame is regulated so that the liquid boils gently in the bulb for about fifteen minutes. During this time the steam (as indicated by a visible rise of the condensate or by running the hand along the tube and determining the height of the steam by the temperature) must not be permitted to rise beyond *b*. This is done by properly regulating the flame. The part of the tube from *a* to *c* acts as a reflux for the steam, while the ether vapors rise past *c*, condense and collect in the receiver. After mild boiling has been continued for fifteen minutes, the flame is gradually increased so that the steam is made to rise

slowly (in one minute) until it just passes the bend *c*. Rectification is now completed. The ether, if present, will be found unfrozen in the calibrated receiver. It sometimes happens that water vapor freezes in the tube beyond *c*, clogging it up; back pressure developed by continued heating will force the distillate out through the safety tube *e*. In that case, the flame is removed; then the receiving tube is removed, and the portion of the tube from *c* to *d* is warmed with the fingers. When the ice melts, it will be sucked back into the flask. The receiving tube is replaced and the rectification continued.

When the rectification is completed, as indicated by steam having passed the bend (*c*), the receiving tube is removed from the acetone bath, lightly stoppered and allowed to stand at room temperature until any ice in the calibrated receiving tube melts. The volume of recovered ether (upper layer) is read immediately.

In order to identify this upper layer as ether, the microboiling point is determined. We are convinced that the isolated ether need not be dried for determination of the boiling point. The isolated ether can be sealed in a capillary tube and kept as *corpus delicti*.

*Determination of Boiling Point.*<sup>6</sup>—A capillary tube (fig. 2 *A*), from 1.5 to 2 mm. in diameter, is drawn out into a finer capillary tube at one end (*C*), about 8 mm. in length. The end (*C*) of the capillary tube is introduced into the ether to be tested. By capillary action, ether is drawn into the tube. Care must be taken to allow only a small droplet to enter. The tube is then sealed by bringing the tip of it into the edge of a Bunsen flame for a second. As the walls of the tube are extremely thin, they fuse together almost instantly. Sealing in this way always leaves a bubble of air in the tip of the tube, between the sealed portion of glass and the drop of liquid (fig. 2 *C*). This bubble of gas serves to prevent superheating of the liquid during the determination of the boiling point. The capillary tube (*A*) containing the drop of liquid and a thermometer (*E*) are then mounted on a microscope slide and set up in a beaker (*F*) containing water, together with a stirrer. The temperature of the bath is raised slowly and uniformly. According to Emich, the true boiling point of a pure liquid is the temperature at which the droplet in the capillary reaches the surface of the bath. This is true only, however, if the bubble of gas in the tip of the tube is so small that the effect of its expansion is negligible, and also if the temperature of the bath is raised regularly and uniformly throughout.

A series of determinations were made on the boiling point of pure, dry ether as well as of ether that had been in contact with water for an indefinite period. The boiling points obtained were: pure, dry ether, from 34.8 to 35 C., and moist ether, from 34.7 to 35 C. Since the boiling point of moist ether is practically the same as that of dry ether, it is not necessary to dry a sample of ether before determining its boiling point.

#### RECOVERY OF ETHER FROM TISSUES

Five hundred gram portions of brain tissue and liver tissue were used. Varying amounts of accurately known quantities of ether were added to the tissues. All possible precautions were taken to avoid loss of ether due to vaporization. The method outlined was used to recover the ether which had been added to the tissues. The results are given in table 1.

6. Emich, F.: *Monatsh. f. Chem.* **38**:219, 1917.

TABLE 1.—*Study of Recovery of Ether*

Number	Tissue Used, 500 Gm.	Ether Added, Cc.	Ether Recovered, Cc.	Ether Lost, Cc.	Per Cent Recovery
1	Brain	0.06	0.03	0.03	50
2	Brain	0.06	0.03	0.03	50
3	Brain	0.12	0.05	0.07	42
4	Brain	0.12	0.07	0.05	60
5	Brain	0.15	0.10	0.05	67
6	Brain	0.15	0.09	0.06	60
7	Liver	0.30	0.24	0.06	80
8	Brain	0.30	0.25	0.05	83
9	Brain	0.45	0.37	0.08	82
10	Brain	0.45	0.38	0.07	84
11	Liver	0.60	0.47	0.13	78
12	Brain	0.72	0.63	0.09	88
13	Brain	0.75	0.67	0.08	89
14	Brain	0.90	0.78	0.12	87
15	Brain	0.90	0.74	0.16	82

TABLE 2.—*Analysis of Cases in Which Death Was Due to Ether*

Patient	Comment	Tissue Used, 500 Gm.	Ether Recov- ered,* Cc.	Boiling Point of Isolated Ether
M. R.	Died before fully anesthetized.....	Brain	0.03	....
		Liver	0.01	....
		Lung	None	....
J. P.	Died after a few inhalations of ether.....	Brain	0.09	34.8
		Liver	0.08	34.9
		Lung	0.02	....
E. W.	Died on table; no operation.....	Brain	0.19	28.5†
T. J.	Died on table; no operation.....	Brain	0.50	34.9
		Liver	0.30	34.9
		Lung	0.34	34.7
W. O.	Died on table just after operation.....	Brain	0.11	35.0
		Lung	0.10	35.0
		Liver	0.14	34.6
M. J.	Anesthesia stopped, 15 minutes.....	Brain	0.37	....
G. W.	Anesthesia stopped, 30 minutes.....	Brain	0.21	35.1
		Liver	0.22	34.9
		Lung	0.14	35.0
T. M.	Anesthesia stopped, 70 minutes.....	Liver	0.11	35.0
		Lung	0.09	34.5
W. W.	Anesthesia stopped, 75 minutes.....	Brain	0.09	....
J. L.	Anesthesia stopped, 85 minutes.....	Brain	None‡	....
R. F.	Anesthesia stopped, 4½ hours.....	Brain	None	....
L. A.	Anesthesia stopped, 7½ hours.....	Brain	None	....
L. J.	Anesthesia stopped, 16 hours.....	Brain	None	....
R. R.	Anesthesia stopped, 20 hours.....	Brain	None	....

\* This is approximately 84 per cent of the amount of ether actually present in 500 Gm. of tissue.

† This ether contained ethyl chloride, hence the low boiling point. In cases of this kind the ether is separated from the ethyl chloride by fractional distillation.<sup>2</sup>

‡ This does not mean that no ether was present. It simply means that probably less than 0.03 cc. of ether was present.

Table 1 indicates that by our method as little as 0.06 cc. of ether in 500 Gm. of tissue can be isolated. It shows further that if tissues contain about 0.3 cc. or more of ether in 500 Gm., the yield of isolated ether is on the average 83.6 per cent ( $\pm 5$  per cent). If the ether present in the tissues is very small (cases 1 to 6), the percentage yield is much smaller (from 42 to 60 per cent). It will be noticed, however, that the ether lost in the procedure is extremely small (from 0.03 to 0.07 cc.). This loss is relatively large on the basis of percentage. In cases in which the patient dies under full ether anesthesia or as a result of an accidental overdose of ether and in homicides by the administration of ether, the ether in the tissues is always higher than 0.3 cc. in 500 Gm. Therefore, in such cases the yield is good and fairly constant. The direct measurement of the isolated ether can be used for the estimation of the ether actually present in the tissues.

The data in table 2 prove that the ether (in excess of 0.03 cc.) present in tissues as a result of anesthetization can be isolated and measured. Recovered ether in amounts as small as 0.03 cc. can be used for the determination of the microboiling point. The boiling points obtained on the specimens of isolated ether ranged from 34.5 to 35.1 C. These values compare favorably with the theoretical boiling point of ether, from 34.7 to 35 C. The boiling point, therefore, can be used for identifying the ether. Other properties of identification of ether are: It is a thin mobile liquid; it is lighter than water, does not freeze in a solid carbon dioxide-acetone bath, has an ethereal odor, takes fire rapidly and burns brilliantly.

We are drawing no conclusions as to the amount of ether in the brain when a patient is fully anesthetized, because the data are still too meager. Further research is in progress on this phase.

#### SUMMARY

A micromethod for isolating ethyl ether from human tissues is described. Amounts of ether as small as 0.06 cc. in 500 Gm. of tissue were isolated and measured. In tissues containing from 0.3 to 0.9 cc. of ether in 500 Gm., the recovery is 83.6 per cent (average). The recovery of ether is fairly constant and can be used for estimating the amount originally present in the tissues. Determinations of the microboiling point served to identify the isolated liquid as ethyl ether.

# HISTOLOGIC CHANGES IN THE KNEE JOINT WITH ADVANCING AGE

RELATION TO DEGENERATIVE ARTHRITIS

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In a previous article, we<sup>1</sup> discussed the gross anatomic changes observed in one hundred knee joints removed from patients who had died of various diseases. It was stated that erosions occurred with increasing frequency with advancing age, and that the alterations were precisely the same as those previously described by many observers as characteristic of degenerative arthritis.<sup>2</sup> In order to determine whether the anatomic changes were the same histologically as those frequently described in degenerative arthritis, the abnormal areas, the synovial membrane and the capsule were examined microscopically.

Material for histologic study was taken from the synovia, cartilage and bone. Approximately one thousand microscopic sections from these joints were examined. A summary of the results is recorded.

## THE SYNOVIAL MEMBRANE

On opening the knee joint, the synovial membrane appeared smooth and glistening in 66 per cent of the cases. The cavity of the joint usually contained a small amount of fluid. In a few instances the synovial cavity of the knee joint communicated directly with that of the tibiofibular joint. This is of practical significance, as an infection of one joint may extend into the other through this direct communication.

As a rule, the synovial membrane showed gross abnormalities only when there were striking alterations in the cartilage and bone. The

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From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), the Department of Pathology, Boston City Hospital, and the Department of Medicine, Harvard Medical School.

1. Keefer, C. S.; Parker, F., Jr.; Myers, W. K., and Irwin, R. L.: Relation-ship Between Anatomic Changes in Knee Joint with Advancing Age and Degenerative Arthritis, *Arch. Int. Med.* **53**:325 (March) 1934.

2. In this report, the term "degenerative arthritis" is used synonymously with osteo-arthritis or hypertrophic arthritis.

most common observation was that of papillary projections extending from the surface of the synovial membrane. These were especially numerous and prominent about the edges of the patella, and inasmuch as erosions of the cartilage were more common on the medial than on the lateral patellar facets, the synovial fringes were seen more often at this location. In some cases, these fringes completely filled the depressions caused by erosions; in others, they projected from the surface into the joint cavity, particularly about the region of the semilunar cartilages. In the cases in which the fatty pad was large and abundant, the synovia over the surface projected in small nodules or in fringes, filling in every small part of the joint cavity.

The capsule of the joint was, as a rule, not thickened, although in the cases in which the capsule was stretched the fibrous tissue was extremely tough and of increased density.

Histologically the synovial membrane consisted of loose connective tissue containing elastic fibers and numerous groups of fat cells. Over the surface, the cells were flattened, the thickness of the surface layer being only two or three cells deep. These surface cells were irregularly oval or stellate and closely packed together. The capsule was composed of interlacing thick bundles of dense fibrous tissue which varied in thickness in the different joints. Blood vessels and nerves were found in abundance in the synovia, and some of the nerve fibers had pacinian corpuscles as endings. Lymphatics were likewise seen within the synovial membrane, usually just below the surface.

We have said that in some areas there were projections of the synovial membrane into the joint cavity. They consisted of connective tissue, capillary blood vessels and masses of fat and were covered with layers of synovial cells. In some the surface cells were heaped up in several layers beneath which were many lymphocytes in the connective tissue core. In a few there were perivascular collections of lymphocytes and rarely focal collections of plasma cells. The core in a few of the projections was hyalin in appearance or contained fibrous tissue of variable density.

In the subsynovial layer there were a few lymphocytes, a rare mast cell or a few plasma cells. In a few sections one could see blood pigment in mononuclear cells and dense collagen about the blood vessels. In the larger blood vessels of the joint capsule one frequently observed intimal thickening. The smaller vessels, as a rule, did not show any abnormal anatomic changes, although in a few cases thrombi were observed in the smaller vessels with a few polymorphonuclear cells surrounding them. The case in which obliterative changes were shown in the smaller blood vessels were usually those in which there were stretching of the capsule and extensive anatomic changes in the joints.

Metaplasia of the connective tissue of the synovia and capsule was observed with resulting cartilage and bone formation at various stages of development. This is shown in figure 1.

#### THE ARTICULAR CARTILAGE

The earliest change was a decrease in basic staining with a tendency toward staining with acid dyes. Associated with this was a development

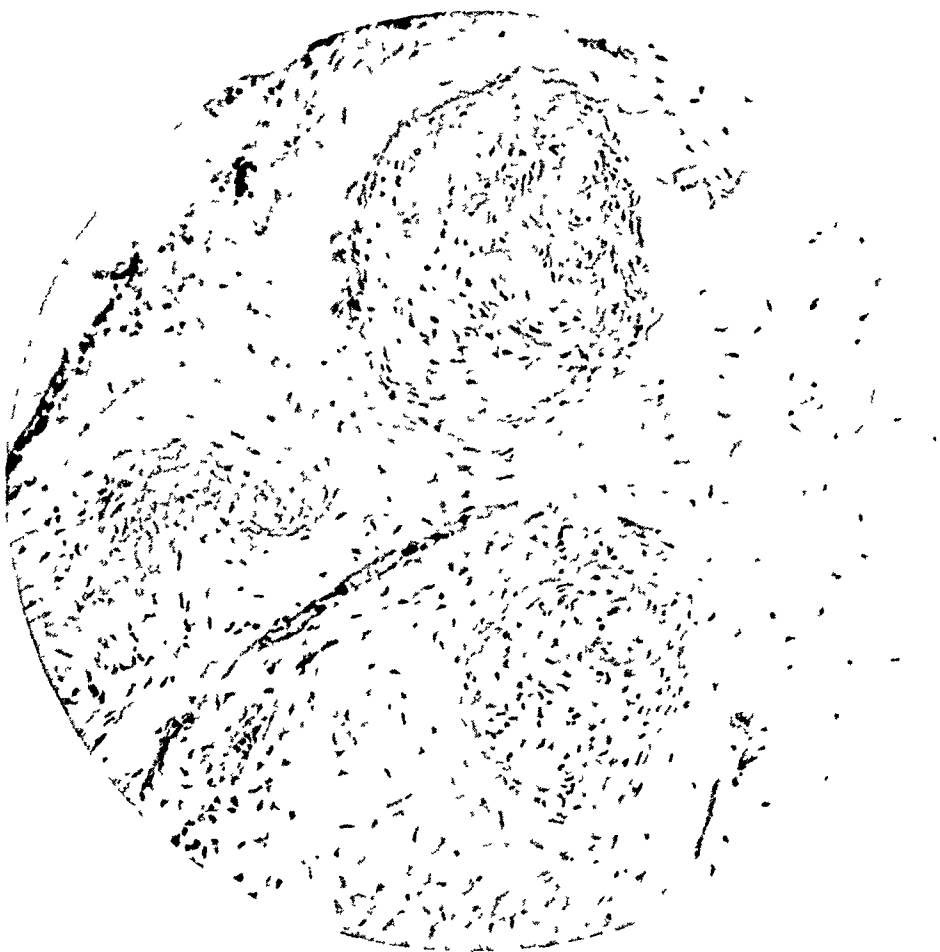


Fig. 1.—Metaplasia of synovial membrane. Islands of young cartilage cells in the synovial membrane;  $\times 110$ .

of fissures resulting in an irregularity of the surface with papillary projections. In such projections there were roughly spherical groups of ten or more small, young cartilage cells. This process was at first superficial, but later the fissures extended deeper into the cartilage, and here the cells appeared fewer and swollen. With loss of the superficial layers and extension of the process to the subchondral bony plate the normal arrangement and appearance of the deep cartilage cells were lost, and only groups of the small cartilage cells heretofore described were present. Finally, there was complete destruction of the cartilage

with exposure of the subchondral bone. Occasionally the cartilage showed cystic areas filled with mucoid material; in several instances at its junction with the bony plate it contained no nuclei, was granular and definitely necrotic. In such areas erosion of the more superficial layers had occurred. Sometimes when there was erosion of the cartilage the denuded areas were covered with somewhat vascular connective tissue continuous with that of the marrow. Embedded in this tissue were chondroid tissue and fragments of necrotic cartilage surrounded by foreign body giant cells. In rare instances, the connective tissue over the uncovered bone was seen as an ingrowth from the capsule.

Where the bone and cartilage had been depressed below the normal line of the articular surface the cartilage had thickened to fill in the defect, with resulting approximate restoration of the joint line.

#### CHANGES IN THE BONE

1. *Subchondral Bone*.—The subchondral bone showed increased thickness in areas which were the site of erosions of the articular cartilage, and also where the cartilage and bone had been depressed below the normal articular surface. This bony thickening was due to compression, as indicated by the distortion of the trabeculae, and to formation of new bone. In the areas of thickening the cartilage extended into the bone more deeply than normally. The prolongations resembled normal ones so far as the cartilage was concerned and were quite different from the islands of cartilage subsequently described. In these areas, the blood vessels showed slightly increased perivascular connective tissue, often with slight infiltration by lymphocytes.

In cases in which the destruction of the articular cartilage was marked with frequently accompanying fragmentation of the cartilage and necrosis there was also necrosis of the bone. In some of the lesions of this type new bone was forming and, as a rule, there was a marked increase of connective tissue in the marrow extending up to and over the affected surface (fig. 2). Occasionally small longitudinal fractures of the bony plate occurred with an extension of the articular cartilage between the fragments. In the subchondral bone cysts filled with a mucoid material were not uncommon.

2. *Islands of Cartilage*.—Associated with marked lesions of the articular cartilage, islands of hyaline cartilage were found in the subchondral region. These islands were situated both within the bony trabeculae and on their surfaces. Those within the trabeculae were often irregular; in some instances they were connected with the articular cartilage while in others they were located at some distance from the articular cartilage and showed no apparent connection. The cartilaginous islands on the surface of the trabeculae were, as a rule, roughly



spherical and attached to the bone at one point. They apparently arose in the endosteal layer, for in the earliest stages of their formation a single cartilage cell could be found in this location. The cartilage of which these islands were composed was of the hyaline type with well formed young cartilage cells. Some of the islands on the surfaces of the trabeculae were undergoing ossification, a layer of new bone sur-

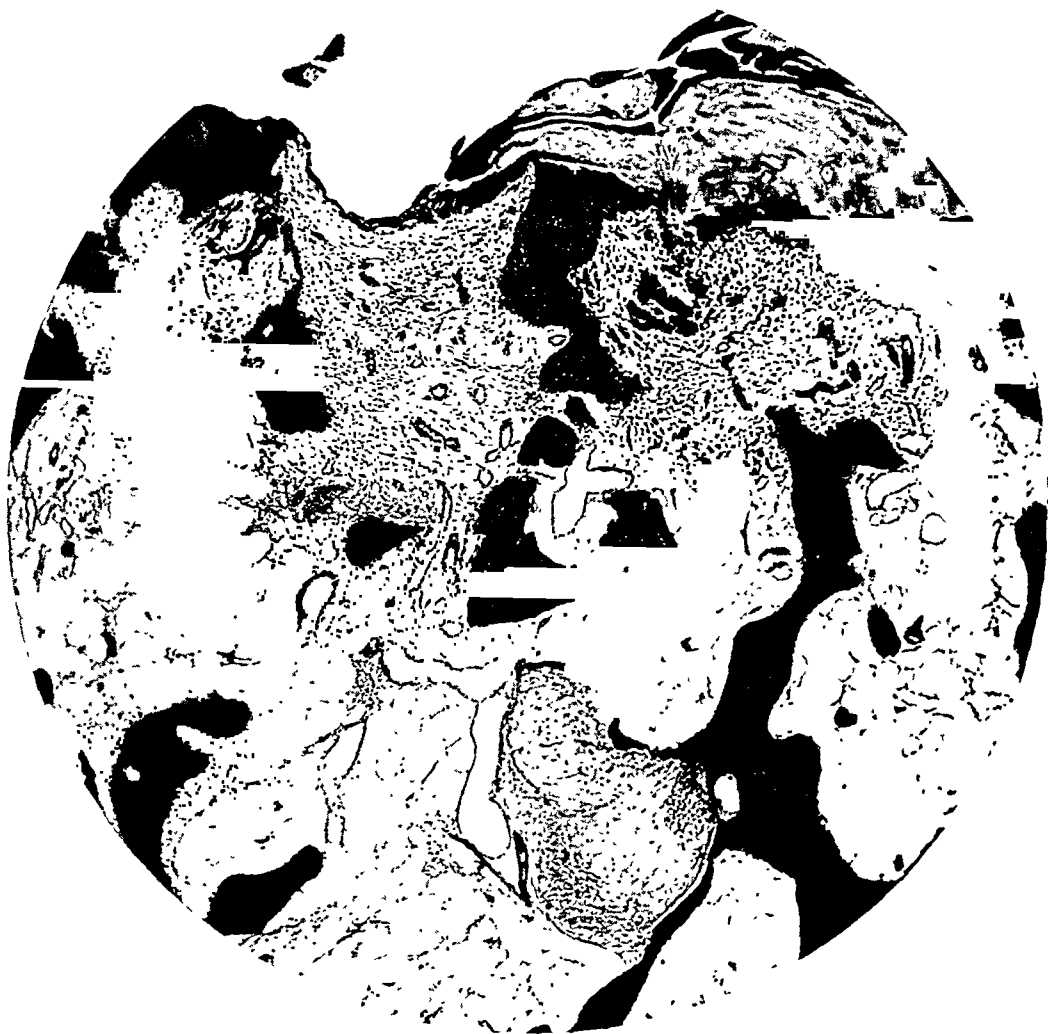


Fig. 2.—Island of cartilage in bone marrow pushing aside and compressing the blood vessels. Erosion of cartilage and bone with areas of connective tissue proliferation and new blood vessels in the subchondral area. Formation of new cartilage and bone is present in the subchondral area;  $\times 50$ .

rounding the cartilage on the side toward the marrow; there was no central ossification such as occurs in exostosis cartilaginea.

The islands in the marrow not connected with bone or with endosteum arose from a metaplasia of the connective tissue of the marrow, a process similar to that noted in the synovia. The earliest stage was a thickening of the connective tissue with the appearance of a few

small cartilage cells embedded in a pink-staining, hyaline matrix. Later these cells increased in size and were surrounded by a dense, hyaline, basophilic staining substance—chondromucin (fig. 3). At the periphery of such an island containing well developed cartilage cells were young cells similar to those described as appearing first.

3. *Formation of New Bone*.—Formation of new bone was found both at the articular surface and in the subchondral trabeculae. It



Fig. 3.—Young cartilage cells arising from connective tissue of the bone marrow. They are not attached to the endosteum of the trabeculae;  $\times 110$ .

occurred at the former site when there had been destruction of the cartilage and bone and was usually accompanied by a proliferation of vascular connective tissue arising in the marrow.

The subchondral bony plate and the underlying trabeculae showed the formation of new bone at the base of erosions and of the aforementioned depressed areas. This bone was formed by the laying down of new bone along the surfaces of the old bone and by the ossification of the islands of cartilage. Wherever the process was active the marrow spaces were filled with vascular connective tissue.

The formation of new bone took place in still another way. In the subchondral bone, extending upward into the overlying cartilage, there could be found islands of vascular connective tissue. Some of these merely consisted of this tissue with osteoclasts lying in its interstices. Others had a peripheral row of osteoblasts while, in still others, ossification of the adjacent cartilage was taking place with the result that such foci of vascularization were partly or entirely surrounded

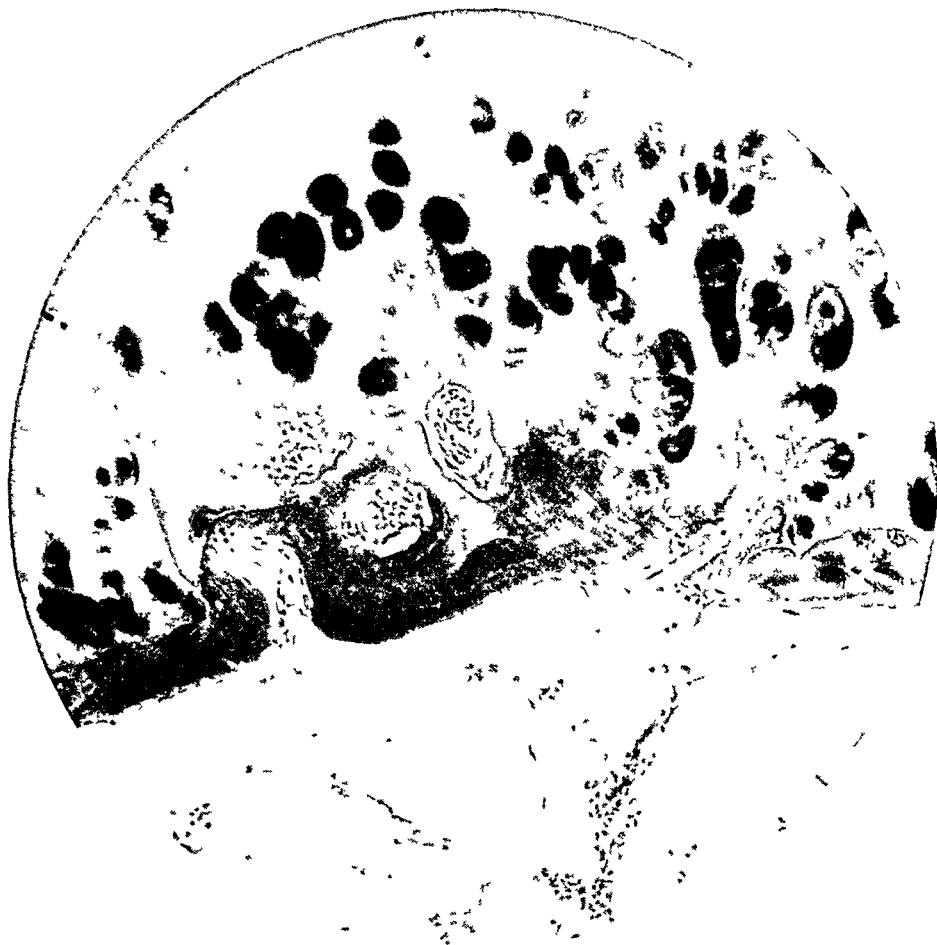


Fig. 4.—Vascularization of the subchondral area. Formation of new bone about one of these areas;  $\times 110$ .

by bone. As a rule, this new bone was at first unconnected with the subchondral plate; but it eventually became connected with it, appearing finally as a roughly spherical or hemispherical, avascular nodule. The end-result was that the subchondral bony plate showed an irregularly nodular surface instead of the normal smooth line.

In several instances the vessels in these islands of vascularization could be followed into the marrow, and there they lost their sheath of connective tissue (fig. 4).

4. *Fractures*.—Several instances of fractures of the articular cartilage and subchondral bony plate were found. In the most marked case, one portion of the cartilage and bone had overridden the other. The cartilage in both portions was in good condition. Formation of new bone was active on the articular aspect of the depressed cartilage and on the under surface of the subchondral bony plate of the overriding part. The cartilaginous parts of the two portions had made a firm union, leaving no visible line of separation. The joint surface showed a U-shaped depression over this area.



Fig. 5.—Fracture of the subchondral bony plate with articular cartilage forced into the area of fracture. Fragmentation of surface cartilage:  $\times 50$ .

In several cases, the subchondral bony plate showed small longitudinal fractures with protrusion of the cartilage into the underlying marrow. This condition is illustrated in figure 5.

In one specimen there was a projection of bone in one area above the line of the subchondral bony plate. The articular cartilage was missing and the surface was covered with connective tissue. The bony trabeculae were thickened and the marrow spaces filled with rather dense fibrous tissue. This lesion was interpreted as representing a previous longitudinal fracture with bony repair.

5. *Marrow*.—Where erosion of the articular cartilage had occurred, extending down to and often into the subchondral bony plate, there was,

as a rule, a proliferation of connective tissue in the marrow spaces beneath the eroded areas. This connective tissue at first appeared as a delicate reticular tissue and was usually infiltrated with lymphocytes and a few macrophages. An atrophy of the fat tissue accompanied its appearance. As this connective tissue became older, it grew denser and contained numerous well formed blood vessels. It appeared first in the marrow spaces beneath the subchondral plate, but if the erosion

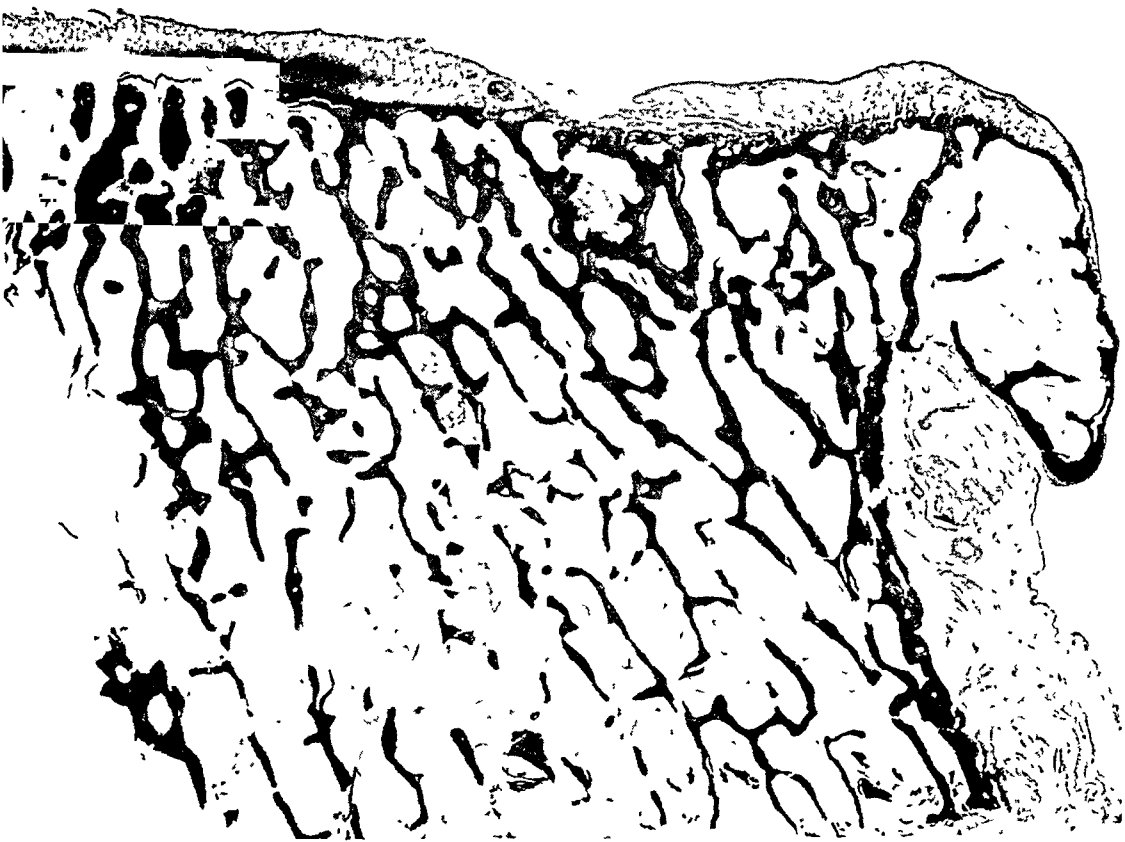


Fig. 6.—Articular surface of the femur showing thinning and fibrillation of the cartilage, flattening of the joint surface and formation of a bony projection ("exostosis") due to forcing of the bone outward;  $\times 25$ .

was extensive and involved the bone, it extended up to and over the articular surface as well as deeper into the marrow. Not infrequently fragments of dead bone and cartilage could be found embedded in the superficial layers. Cysts occurred in the deeper portions. The fatty tissue underwent atrophy as the connective tissue proliferated and also in certain instances degenerated and became necrotic as evidenced by collections of macrophages arranged peripherally around the fat cells.

Rarely was any blood-forming tissue present in the marrow; when it occurred, it was in small amounts.

Vascular changes, that is, intimal thickening, were frequent, but no specific changes in the surrounding tissue could be associated with them.

6. *Exostoses*.—A considerable number of our specimens in which there was flattening of the joint surface showed projections at their peripheries. These projections consisted of fatty marrow, trabeculae of bone and a layer of bone continuous with the subchondral plate (fig. 6). They were partly covered with articular cartilage and partly with connective tissue which could be followed into the capsule of the joint.

The marrow was essentially normal, although at times it showed infiltration with a few lymphocytes. No proliferation of connective tissue and no islands of cartilage were noted in the marrow of the projections.

The bony trabeculae were made up of well formed, dense bone. Their long axes were either parallel with or at right angles to those of the trabeculae of the epiphysis. No evidence of a formation of new bone was present.

The covering layer of bone was, as aforesaid, a continuation of the subchondral plate. In a number of instances, however, there was evidence of a formation of new bone and a destruction of bone in this peripheral region. The new bone was for the most part formed on the endosteal side, but sometimes the periosteum also took part in the process.

The cartilaginous portions of these projections were continuous with the articular cartilage and resembled the latter in every respect.

Occasionally, the projections were vertical, that is, at right angles to and projecting above the line of the joint surface, but usually they were parallel to the articular surface and in shape suggested a rolled-over edge of the joint surface.

Islands of bone and cartilage occurred occasionally in the connective tissue of the joint capsule and in the attachments of the tendons.

#### COMMENT

It has been repeatedly shown that the characteristic lesions in degenerative arthritis are: (1) changes in the cartilage such as fibrillation, erosion and destruction with attempts at regeneration; (2) alterations in the subchondral bone due to increased vascularization, ossification, fracture, attempts at repair with formation of new bone, islands of cartilage and cysts in the cancellous bone, and (3) the formation of exostoses, sometimes called lipping, "Randwülsten," etc. The term "hypertrophic arthritis" has been applied to this condition on account of the increased density of the bone and overgrowth of the cartilage. We believe that these processes result from the repair occurring in damaged cartilage and bone and are not primary lesions

of the joints. We have arrived at this conclusion as a result of the study of the gross and microscopic changes in the joints detailed in the foregoing sections. Inasmuch as it is important to establish whether these changes are primary or whether they result from attempts of the parts to repair the damage done by wear and tear, the changes we have observed require special comment.

*Changes in the Cartilage.*—In the gross description of the joints, we described the early changes observed in the cartilage. These changes were characterized by a fragmentation of the cartilage in the vertical plane so that the surface had an irregular and uneven appearance, resembling the pile of a woolen rug. Following this fibrillation, the cartilage was frequently worn away so that the underlying subchondral bone was exposed. Often one observed evidence of regeneration of the cartilage, but at best this was not striking, probably owing partly to the poor regenerative powers of cartilage and partly to the movement which takes place in such joints. Many of the areas of cartilage showing regeneration were similar to those observed experimentally in animals by Bennett, Bauer and Maddock,<sup>3</sup> thus demonstrating that hyaline cartilage may regenerate in man as well as in animals.

*Islands of Cartilage.*—In common with other observers, we have found islands of cartilage cells in the marrow at some distance from the joint surface. The origin of these cartilaginous areas has been the subject of many controversial discussions. As a result of a study of many cases, Pommer<sup>4</sup> and Lang<sup>5</sup> concluded either that they were due to emboli of cartilage cells which were carried to the marrow by the blood vessels or lymphatics or they arose from microscopic fractures in the cancellous bone. Heine<sup>6</sup> expressed the opinion that many of these islands arose from cartilage that had been forced “durch Einpressung” into the subchondral bone.

Both Pommer and Lang based their conclusions on the observation that these islands were found in the lumens of canals, in the callus that followed fractures of the subchondral bone and in areas that they interpreted as being within blood vessels or in the perivascular lymphatics.

We have considerable anatomic material bearing on the origin of these islands of cartilage as they were seen in the sections from the knee joints. We observed that in the areas where there were fractures in the subchondral plate cartilage was frequently depressed between the edges of the fracture (fig. 5), so that it is possible that in some cases the areas of cartilage in the subchondral bone have such an origin.

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3. Bennett, G. A.; Bauer, W., and Maddock, S. J.: *Am. J. Path.* **8**:499, 1932.

4. Pommer, G.: *Virchows Arch. f. path. Anat.* **263**:434, 1927.

5. Lang, F. J.: *J. Bone & Joint Surg.* **14**:563, 1932.

6. Heine, J.: *Virchows Arch. f. path. Anat.* **260**:521, 1926

However, the islands of cartilage in the subchondral bone arise more often from the endosteal layer of bony trabeculae (fig. 7). This is also true when they are located some distance from the subchondral area. In other cases, islands of young cartilage arising from the connective issue of the marrow (fig. 3) may be found, having no connection with the endosteum. These islands of cartilage may also arise from the connective tissue of the callus which forms following small fractures



Fig. 7—Island of cartilage arising from the endosteum of bony trabeculae of the epiphysis;  $\times 110$ .

in the subchondral area. These islands differ in origin from the ones heretofore mentioned in which the cartilage extends through a fracture of the joint surface. The islands formed from the connective tissue of the callus are situated at some distance from the joint line.

We have examined many sections for evidence that these islands of cartilage develop from small emboli in the blood vessels or perivascular lymphatics as postulated by Pommer. We have not observed any which we could with assurance classify as arising from the blood vessels or



lymphatics. Indeed, in some of the sections islands of cartilage were found developing outside the blood vessels and compressing or pushing aside the vessels as they grew (fig. 2).

From our observations, then, it seems abundantly clear that the islands of cartilage may arise in any one of the following ways: (1) as a result of fracture of the subchondral bone with formation of callus and connective tissue; (2) from the endosteum of the bony trabeculae of the epiphysis; (3) from the connective tissue of the marrow through metaplasia. We were unable to find evidence that they arose from emboli in the blood vessels or lymphatics.

As these islands were observed only in joints in which there was extensive damage to the articular cartilage or to the underlying subchondral bone, it seems fair to assume that they represent a process of repair rather than a primary lesion.

*Exostoses*.—It has long since been generally admitted that one of the characteristic features of degenerative arthritis is the development of exostoses at the margins of the joint surfaces. Indeed, many observers have regarded their presence as necessary before the diagnosis can be accepted. The origin of these bony protuberances has been an occasion of considerable discussion. They have been described as ossified excrescences, as periosteal or perichondrial proliferations, as osteophytes or as exostoses formed by depression and bending over of the tissue at the edge of the joint surface. Pommer, Lang and others have stated that they arise as outgrowths from the subchondral bone, possess a laminated structure and are usually covered with new cartilage. They admit, however, that the periosteum may show bony proliferation covered by new cartilage at the insertions of tendons, and that it is common to find a formation of new bone in the tendon attachments themselves. This condition is illustrated in children in whom there is increased tension of the muscle tendons at the points of attachment to the bone; excrescences occur at those sites. The same phenomenon may be observed in the osteophytes on the ilium in osteo-arthritis of the hip joint. In the knee joint, however, lipping occurs not only at points which are the sites of attachment of muscle tendons but also at the margins of the patella, femur and tibia.

From our observations on the knee joint we have become extremely skeptical as to whether all these exostoses at the margins of the joint surfaces are really the result of a formation of new bone arising from the subchondral space. We have considerable data bearing on this point. When the joint surfaces and the subchondral areas of a normal joint are compared with those of one showing exostoses, several distinctions are evident. In the first place, exostoses are seen in a joint which is the site of damage or in which the surfaces have not been

mutually adapted to one another. The joint surface is frequently flattened. This is especially striking in a joint in which there is a displacement of the patella either medially or laterally. The bony projections often suggest a mushroom and seem to have been forced outward as a result of the flattening of the joint surface. That is to say, as the normal convex surface of the femur is worn away, the cartilage disappears over the area of pressure and movement, the subchondral bone thickens, and the area of pressure becomes flattened, less convex and, indeed, in some cases depressed below the previous normal line. This flattening of the surface which is in contact with the opposing joint surface causes the edge of the bone to project outward as a shelf, and, depending on weight, pressure and position, the projection may curl or bend over at the edge. These bony prominences are not the result of new growth of bone in every instance, although in the subchondral layer of some of the projections slight evidence of a formation of new bone may be seen as in other subchondral areas. The trabeculae of the projections are either continuous with the cancellous bone or, at the sites where they project over the edge, at right angles to it. We have not found a double layer of cartilage, such as that described by Pommer, Nichols and Richardson,<sup>7</sup> to indicate that the projections result from perichondral or subchondral proliferation. We have searched numerous sections for a double row of cartilage cells without success. In examining numerous gross specimens we found several in which the bony projections curled over at the edge. At first glance it might appear that these represented layering such as has been described. On closer inspection, however, it could be shown that this was not the case and that the double layer of cartilage was due to a projection of the bone and cartilage over the edge of the joint surface. It should be emphasized that the cartilage projects over the edge of the joint surface to a considerable extent in some normal joints so that the variations cause differences in the appearance of the projections.

We believe, therefore, that these bony projections, commonly called exostoses, are areas of bone and cartilage occurring at the edge of the joint surface. They become prominent and conspicuous when the surface of the bone has been altered and flattened, so that the bone and cartilage are forced outward and downward. If, on the other hand, there is a depression of the bone near the edge of the joint surface, the peripheral portion of the joint surface may appear to project above the surface. We found no evidence that these lesions were primary, and we regard them as a part of the change that takes place in distorted joints following alterations in the joint surface near the edge.

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7. Nichols, E. H., and Richardson, F. L.: *J. M. Research* 21:149, 1909.

*Subchondral Bone.*—Frequently one of the earliest changes observed in these joints was in the subchondral bone. Pommer was the first to point out that one of the characteristic lesions of degenerative arthritis was an increased vascularization and bony thickening of the subchondral plate. In discussing this phenomenon he stated that the loss of the protection which the subchondral bone received from the cartilage when it lost its elasticity or was destroyed resulted in subchondral thickening with formation of new bone. Nichols and Richardson also described changes in the epiphyseal end of the bone in the cases in which the surface cartilage was destroyed and, also, alterations in the base of the articular cartilage in cases in which the surface was damaged. We have

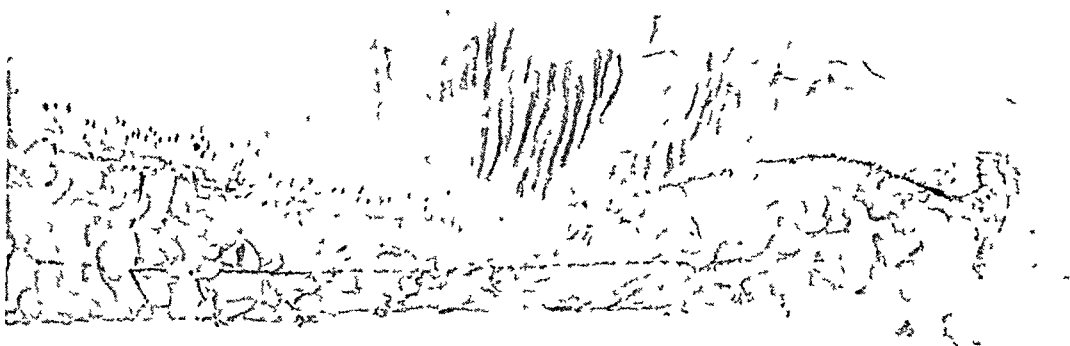


Fig. 8.—Early lesions showing fibrillation of cartilage with compression of the underlying bony trabeculae;  $\times 50$ .

frequently observed that one of the earliest changes that occurs in the subchondral bone is a compression of the trabeculae due to loss of elasticity and fibrillation in the overlying cartilage (fig. 8). In some of the areas of this type there may be little or no evidence of a formation of new bone; in others, a formation of new bone is obvious.

In addition to such bony changes we observed various stages of the aforementioned vascularization. The earliest stage of this process consists in the appearance in the subchondral bony plate of islands of vascular connective tissue. The vascular tissue extends from the bony plate into the adjacent articular cartilage. One gains the impression that the islands at first cause solution of the surrounding bone and cartilage; osteoclasts are often present. At a later stage osteoblasts appear and, associated with these, ossification of the neighboring

cartilage takes place, a narrow layer of new bone being formed around the island. As time goes on the bone becomes thicker, the connective tissue and blood vessels disappear, and finally there remains a nodule of well formed bone. At first, this new bone is usually unconnected with the subchondral plate, but later it fuses with the plate. When such a process has healed, the upper surface of the plate presents a series of nodules of bone projecting into the base of the articular cartilage. It is difficult to determine the mode of origin of these islands. While it is often possible to trace a blood vessel from such an area into the marrow, we do not believe that there is definite proof that the islands arise from invasion of the bone and cartilage by vessels from the marrow. Such vessels, as a rule, do not show in the marrow the sheath of connective tissue which they possess in the bone and cartilage. It would seem equally reasonable to suppose that these vascular islands arise by a proliferation of connective tissue around vessels preexisting in the bone. Accompanying this proliferation there is a solution of the adjacent bone. Then this vascular tissue penetrates the cartilage and later leads to ossification of the cartilage.

Where erosions of the cartilage have occurred with exposure of the bony surface, thickening of the bone takes place owing to formation of new bone. The new bone is formed by being laid down on the old bone and also by ossification of the islands of cartilage mentioned elsewhere. Accompanying the formation of new bone there is a proliferation of connective tissue arising in the marrow and extending to the subchondral plate. Where destruction of bone, as well as of cartilage, has occurred, this connective tissue extends up to the articular surface, thus filling in the defect.

We view these changes in the subchondral bone as a reaction to the loss of elasticity in and damage to the overlying cartilage. It is really a process of repair or an attempt on the part of the bone to strengthen areas subjected to increased pressure. In view of these alterations occurring in joints which are the site of degenerative changes, one should be extremely cautious in interpreting them as a part of the essential pathologic picture in joints which are the site of proliferative processes. More will be said about this matter when we discuss the pathologic changes of proliferative (rheumatoid) arthritis in a subsequent communication.

In view of our studies it is justifiable to say that the changes which occur in the joints with advancing age are similar, both grossly and microscopically, to those that have been described as being characteristic of degenerative arthritis. We feel that the anatomic changes are caused by injury to the various tissues of the joint and that the final anatomic picture is due to a combination of alterations produced by injury and repair. We believe the sequence of events to be as follows: fibrillation

and thinning of cartilage, compression, vascularization with formation of new bone in subchondral bone, formation of fibrous tissue, of islands of cartilage and of cysts in the bone marrow, and formation of bony projections at the edge of the bone owing to alteration of the joint surface forcing the bone and cartilage outward.

#### SUMMARY AND CONCLUSIONS

Histologic examination of the tissues from one hundred knee joints of patients who had died of miscellaneous diseases was made. The following points were brought out.

1. The synovia was essentially normal except in cases in which there were alterations in the cartilage. In these the capsule was somewhat thickened, papillary projections of the synovia occurred, and occasionally there were small collections of lymphocytes about the blood vessels.

2. The cartilage showed fibrillation, degeneration and some areas of regeneration. In some cases it was completely destroyed.

3. The subchondral bone was thickened owing either to compression or to formation of new bone. The marrow spaces were frequently filled with fibrous tissue, cysts and areas of cartilage.

4. Bony projections, or so-called exostoses, were due to a projection of bone and cartilage over the edge of the joint surface, or to a depression of the cartilage below its original level, thus giving the appearance of bony outgrowth. They resulted from a forcing outward of bone and cartilage by flattening of the joint surface owing to pressure and erosion.

5. These changes are identical with those previously described as characteristic of degenerative arthritis. The evidence is that they are the result of injury and repair and that they occur with increasing frequency with advancing age.

# SUBCUTANEOUS TUBERCULOID LESION OF CATTLE

## A MORPHOLOGIC STUDY

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The true character of the so-called skin lesion of cattle sensitive to tuberculin is still obscure. Although it is generally agreed that the histologic structure of many of these lesions has a marked resemblance to that of lesions of tuberculosis, the evidence advanced to this time is by no means sufficient to prove that *Mycobacterium tuberculosis* is the responsible etiologic agent.

Many workers have investigated these lesions, but the majority of the published reports have been concerned with the bacteriologic phase of the problem. Efforts have been made to isolate by cultural methods and by inoculation of animals an organism of etiologic significance, and relatively little attention has been directed to the morphologic aspects of the lesions.

About three years ago a project was outlined for the purpose of making a comprehensive study of the morphology of this condition. It was the intention to secure lesions from cattle in as many different parts of the United States as possible and to include in the study specimens from Canada, Mexico, South America and Europe. When the material was being collected, a paper by Runnells<sup>1</sup> was published in which were recorded the results of a detailed study of the pathologic histology of the so-called skin lesions. Runnells' fifty-six specimens were secured from fifty animals from six widely separated sections of the United States and Canada as follows: seventy-two from Michigan, six from Arizona, eight from California, five from Montana, three from New York, eleven from Iowa and one from Quebec.

Runnells' study seemed to make additional research on morphology superfluous, and the project was abandoned for the time being. However, specimens continued to be received from different parts of the United States, and an opportunity was presented to conduct additional studies on the bacteriologic aspect of the problem. Consequently, because of the amount of data that eventually has accumulated, it seems desirable to make available for others certain conclusions concerning the character of this rather commonly encountered condition.

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From the Institute of Experimental Medicine, the Mayo Clinic.

1. Runnells, R. A.: J. Am. Vet. M. A. 81:173, 1932.

## SOURCE OF MATERIAL

Requests for specimens were addressed to Inspectors-in-Charge of the Federal Meat Inspection Service, Bureau of Animal Industry, United States Department of Agriculture in various parts of the United States, who secured material from animals slaughtered under their supervision. Fifty specimens were secured from twenty-one states as follows: Alabama, two; California, two; Colorado, three; Georgia, one; Idaho, four; Illinois, five; Indiana, one; Iowa, three; Kansas, two; Maryland, two; Michigan, two; Minnesota, four; Nebraska, two; New Jersey, one; New York, six; Oregon, two; Pennsylvania, one; Utah, three; Vermont, one; Virginia, two, and Wisconsin, one. One specimen was received from Dr. C. A. Mitchell, Ottawa, Canada. Attempts to secure material from Mexico, South America and Europe, including the British Isles, were unsuccessful. Whether the subcutaneous nodules appear only in cattle in the United States and Canada is not certain, although correspondents in Continental Europe and the British Isles were of the opinion that such lesions occur seldom if ever in the cattle of that part of the world.<sup>2</sup>

All animals from which the subcutaneous lesions were obtained had previously revealed sensitivity to tuberculin administered intradermally. The respective specimens were removed from the carcasses during post-mortem examination, were placed immediately in a dilute solution of formaldehyde, U. S. P. (1:10), and were mailed to the laboratory. Lesions were secured only from animals which had no other gross lesions which might have been considered tuberculous. With few exceptions the lesions were confined to the subcutis of the lower extremities and were characteristic of the usual entity designated by federal meat inspectors as "skin lesions."

## METHODS

Histologic sections were prepared from each of the lesions and stained with hematoxylin and eosin. Sections from all lesions were also stained appropriately with carbol-fuchsin to reveal the presence of acid-fast bacteria that might be present. In addition, sections from the more representative lesions were stained for reticulum by the method of Pap.<sup>3</sup>

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2. In a brief report by Fischel (*Wien. tierarztl. Monatschr.* **15**:423, 1928) mention is made of the finding of twelve cases of tuberculosis of cutaneous and subcutaneous tissues among a total of 2,100 cattle slaughtered at St. Marx. Smears revealed acid-fast bacillary forms in several of the lesions, and the tuberculous character of the condition was definitely apparent in sections prepared from the diseased tissues. With one exception the regional lymph nodes were not tuberculous, and in only two instances was there tuberculosis elsewhere in the body. It was not mentioned if efforts had been made to determine the etiology of the lesions by culture or by inoculation of animals, so the exact type of the lesions reported by Fischel remains a matter of conjecture.

3. Pap. Tibor: *Centralbl. f. allg. Path. u. path. Anat.* **47**:116, 1929.

## PATHOLOGIC ANATOMY

*Gross Appearance.*—Few of the lesions obtained actually involved the dermis. The irregular nodular masses, which varied in size from less than 1 cm. to as much as 4 cm. in diameter, were definitely situated in the corium and underlying subcutaneous tissues, often extending from the epithelial layer to the musculature. One somewhat unusual specimen consisted of a compact, elongated mass of granulomatous tissue 3 cm. thick and approximately 20 cm. long. Many of the nodules were irregularly spherical, and others were of a flattened, ovoid shape. All were invested with a tough, indistinct, fibrous capsule which provided a rather firm attachment to the adjacent tissues. On incision mineral salts were revealed in thirty-three of the fifty-one specimens studied. The amount of calcification was variable, but was often sufficient to require decalcification of the tissue before sections could be prepared.

The lesion proper varied in appearance. Some lesions consisted of single, purulent or caseous yellowish abscesses, with dark, hemorrhagic, peripheral zones, but the majority of the lesions were composed of proliferative elements the continuity of which was often interrupted by multiple necrotic foci. The necrotic foci of some nodules were small and extremely numerous, whereas in others two or three foci constituted the lesion. Occasional lesions were observed in which necrosis could not be detected with the unaided eye. These were characterized by appearing to be composed of proliferating tissue elements not unlike new growths. The necrotic content of the various foci varied from a semidry, caseous material to a material that was pasty or even purulent.

*Pathologic Histology.*—Histologically, the material studied could be separated into two general groups. In one group there was a monocytic and epithelioid reaction such as to justify the presumption that a common or closely related provocative agent was responsible for the morphologic changes. These lesions were tuberculoid, with a resemblance to the lesions of genuine tuberculosis that was remarkable in some instances. The lesions constituting the second group were frankly pyogenic, with nothing in common with those in the first group except the situation in which they occurred. Thirty-four of the specimens were tuberculoid, and fourteen were considered nonspecific pyogenic abscesses. Two of the specimens revealed only hyperplasia of the corium without any specific changes, and one of the nodules was diagnosed histologically as an adenoma of the sebaceous glands (adenoma sebaceum).

Since the central portion of the lesion often has a tendency to undergo necrosis, it is imperative that the character of the cells at the periphery be taken into account in studying the histopathologic characteristics of the disease. Although the bulk of the structure may have undergone



necrosis, and few intact cells may remain, there is usually a narrow peripheral zone in which cells characteristic of the lesion can be observed.

The lesions to which I have referred as tuberculoid are subject to considerable variation in histologic appearance, but all have a peripheral zone of monocyctic or epithelioid cells which are of the greatest significance in recognizing a given lesion.

In general, the structural design is of two types, and although a given subcutaneous nodule may reveal exclusively one or the other, it is not uncommon to find in some lesions both varieties of morphologic expression.

In one variety of lesion the histologic picture is strikingly reminiscent of the typical lesion of tuberculosis. The tubercle-like structures occur as single or multiple, lobulated units with a peripheral zone of connective tissue (figs. 1 and 2). The cellular elements of such a structure consist of a predominance of monocyctic cells which often reveal a tendency to assume an epithelioid appearance. The epithelioid cells are more numerous toward the center of the lesion, where necrosis is commonly a prominent feature. The necrosis is usually caseous. In the outer zone of a tubercle of this kind variable numbers of lymphocytes occur, and numerous small vascular channels are not uncommon. Giant cells of the Langhans type are present, often in abundance, in the majority of lesions.

The histologic appearance of the lesions just described is subject to considerable variation, depending apparently on the duration of the disease. Not infrequently an aggressive, proliferative type of the disease is encountered in which the nodules of monocyctic cells are small and extremely numerous, with but little if any necrosis apparent. Mineral salts are often discernible in lesions in which there is considerable necrosis, but may be absent.

In contrast to the multiple, well defined, tubercle-like structures that characterize the lesions just described, considerable numbers of lesions occur in which the pathologic characteristics are somewhat different. The difference, however, is one of dimension rather than of the cellular elements involved. Lesions of the latter type are characterized by the occurrence of extensive, irregular, more or less diffuse areas of necrotic debris, surrounded by a rather narrow peripheral zone of well differentiated epithelioid cells supported by variable amounts of connective tissue, many of the strands of which extend for a considerable distance between the epithelioid cells at right angles to the peripheral capsule (fig. 3). In some instances the necrosis of the lesion has advanced so as to include practically all of the cellular elements within the surrounding capsular investment, and calcification is often excessive. Giant cells of the Lang-

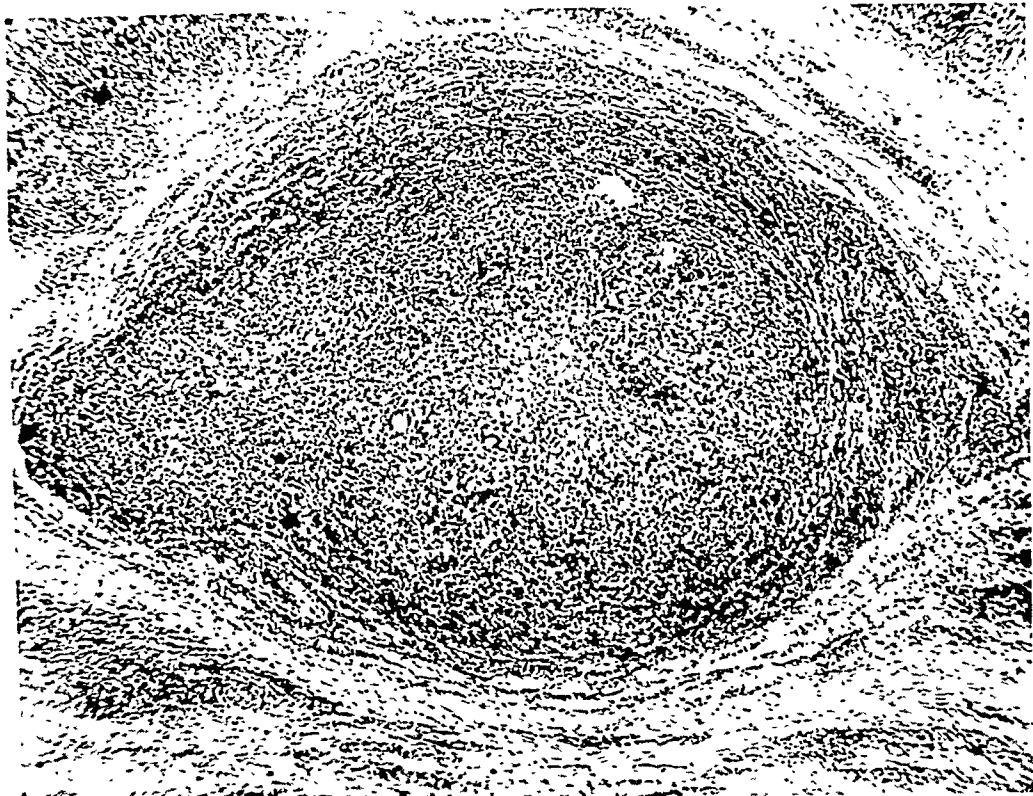


Fig. 1.—Tubercle-like lesion of the subcutaneous tissues with beginning necrosis of the central portion;  $\times 45$ .



Fig. 2.—Small early proliferative tuberculoid lesion composed largely of monocytic cells with peripheral lymphocytic infiltration;  $\times 130$ .

hans type, which are rather numerous in the tubercle-like lesions first described, are rare in the larger and more necrotic variety of lesion.

Sections stained by Pap's method revealed an elaborate reticular system as an integral part of the respective cellular reactions (fig. 4). The reticulum fibrils were most numerous in those tubercle-like units which were in a progressive state. In the larger lesions, in which considerable central necrosis had occurred, the reticulated meshwork had disappeared. The reticulum was best observed in the smaller tubercle-like structures in which there was an abundance of epithelioid cells.



Fig. 3.—Diffuse monocytic and epithelioid cellular reaction with extensive necrosis of a lesion from the subcutaneous tissues;  $\times 45$ .

Here the intricate meshwork of the extremely fine, threadlike, tapering fibrils was indeed striking. The fibrils extended in every direction, but seemed to have their origin in cells near the periphery. These structures were never seen in areas of necrosis even in the smallest tubercle-like units, and were not observed within giant cells of the Langhans type. In some lesions well developed reticulum fibrils could be seen surrounding each epithelioid cell, whereas in others the fibrils surrounded groups or packets of several cells. The reticulum was not unlike that seen in the tubercles of true tuberculous infections of other tissues.

In no instances in the material examined did the proliferative and necrotic process extend into the substance of the musculature, although lymphocytic infiltration frequently was observed between the muscle fibers adjacent to the lesions in the subcutaneous tissues. As a consequence of the increased pressure, many of the muscle fibers in the zone immediately adjacent to the tubercle-like formations were compressed and atrophic.

The changes involving the blood vessels, mentioned by Carpenter and Goldberg,<sup>4</sup> and by Runnells, were often present in the material

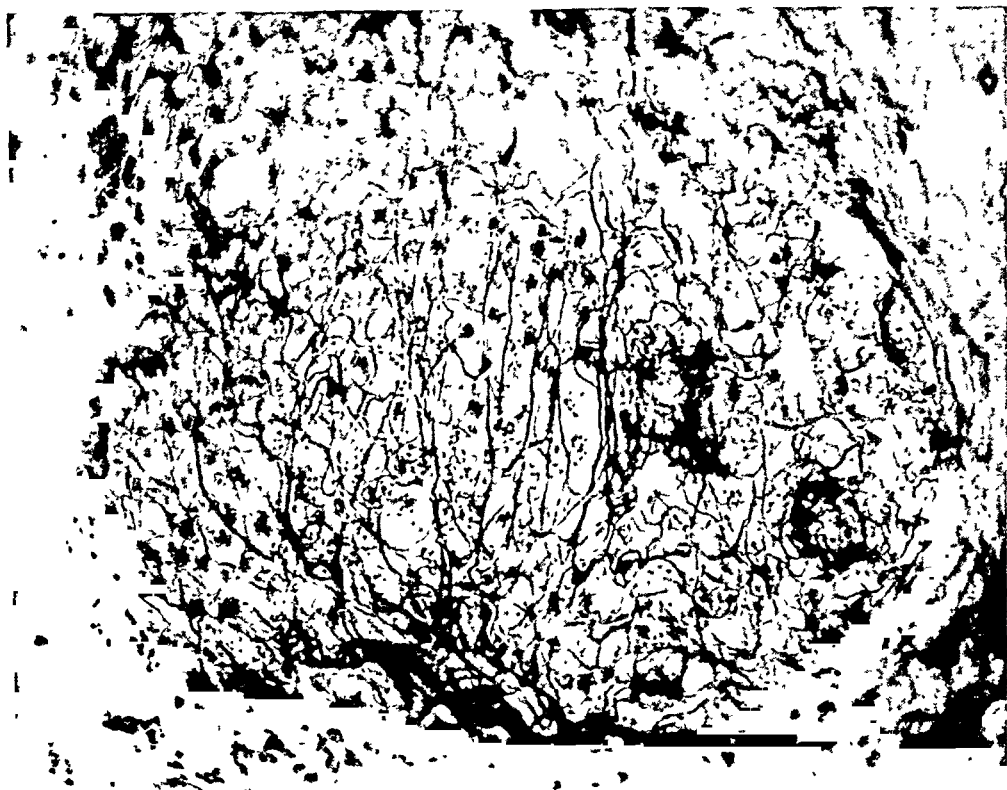


Fig. 4.—Epithelioid tubercle-like reaction stained to reveal the fibrillar reticulum; subcutaneous lesion;  $\times 440$ .

studied. These consisted of perivascular infiltrations, particularly noticeable around the smaller vessels in the corium (fig. 5). The cellular elements were largely monocytic, with few lymphocytes noticeable. It was not uncommon to observe well established changes affecting the lining of blood vessels. The proliferative endarteritis, affecting the entire intima, caused marked narrowing of the lumen, and not infrequently almost complete obliteration of the lumen resulted. A few vessels were observed that contained canalized, thrombus-like obstruc-

4. Carpenter, C. M., and Goldberg, G. A.: *Cornell Veterinarian* **15**:148, 1925.

tions. The character and extent of the vascular changes in much of the material studied were such as to convince me of the significance of the vascular tracts in the genesis of the ultimate tuberculoid lesions. It seems logical to believe, as Runnells pointed out, that the provocative agent entered the subcutaneous tissue through the dermis and followed the perivascular tissue to the depths of the corium and beyond.

Acid-fast bacillary forms were seen in twenty-two of the thirty-four specimens that were considered tuberculoid. The organisms, when

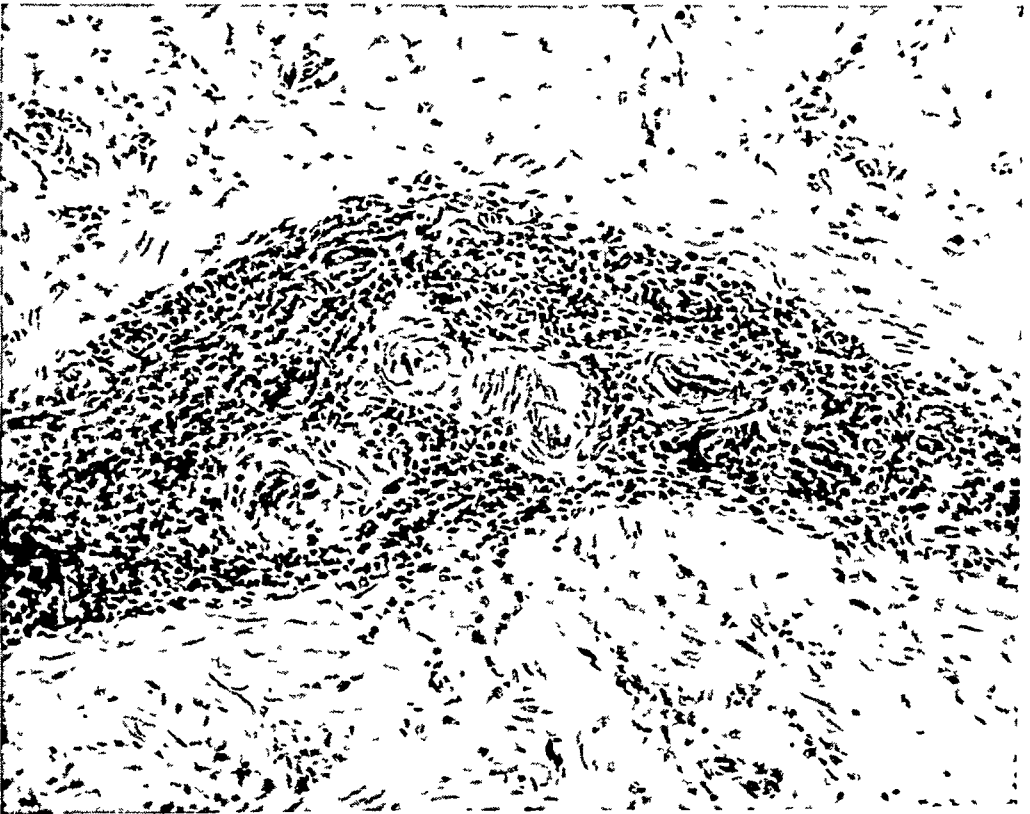


Fig. 5—Perivascular reaction consisting largely of monocytic cells and lymphocytes. Thickening of the vascular walls and narrowing of the lumens may be noted;  $\times 150$ .

present, were discernible usually among the cellular débris of the necrotic portion of the lesion. In the aggressive or actively proliferative tissue of the lesion, acid-fast bacteria were seldom numerous and were generally found with difficulty if at all. The organisms varied greatly in number and somewhat in form. In some preparations only two or three bacteria could be found after prolonged search, whereas in others acid-fast forms were exceedingly numerous (fig. 6). There was no question about their acid-fastness, although often shadowy, nonacid forms could be seen lying in the cellular débris. The acid-fast bacteria often have a beaded appearance, with polar and metachromatic granules retaining the fuchsin stain while the vacuolar areas are unstained.

From the characteristic cellular changes of these lesions, the development or pathogenesis of the condition would seem in general to be about as follows: The provocative agent penetrates the dermis through a break in the continuity of the surface portions of the integument. Once in the corium the infective agent, if in proximity to blood channels, incites a cellular reaction composed largely of mononuclear cells, lymphocytes and a few polymorphonuclear leukocytes. This occurs in the tissue elements immediately adjacent to the adventitia in which progenitors of the monocytic cells are found, although some such cells undoubtedly are derived from the blood stream. As the organisms multiply, the cellular

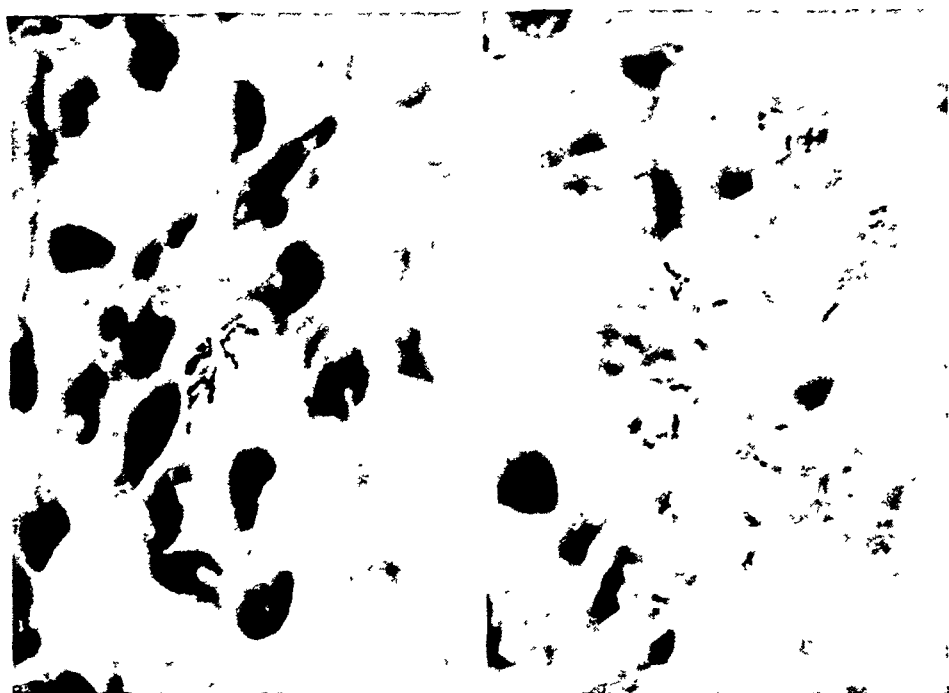


Fig. 6.—Acid-fast bacillary forms in a tuberculoid lesion. Many of the bacteria have a beaded appearance;  $\times 1,350$ .

reaction progresses along, and peripheral to, the vascular channels until the deeper and less resistant subcutaneous tissues are reached. Here the process is continued, with the smaller vascular channels finally disappearing as a consequence of atrophy from pressure and eventual necrosis of the morbid process. In the meantime many of the monocytic cells assume an epithelioid appearance, and fibrous connective tissue is formed at the outer zone of the proliferating cells. This, in turn, eventually becomes granulomatous, with numerous blood vessels and an infiltration of lymphoid cells. The giant cells of the Langhans type which are often present in considerable numbers in the lesions become evident as a consequence of the fusing of mononuclear cells constituting the

primary cellular reaction. The central portions of the lesions, which are avascular and subjected to the noxious influences of the infective agent, become necrotic and calcification follows.

The pathologic change of the nontuberculous diseases was a simple pyogenic abscess in which the salient features were a single encapsulated, or several diffuse, areas of necrotic substance surrounded by fairly vascular granulation tissue. Some abscesses contained mineral salts and others did not. Occasionally giant cells were present, but the peripheral monocytic and epithelioid reaction characteristic of tuberculous lesions was absent. Likewise, acid-fast bacteria could not be demonstrated.

The lesions which were later found to be pyogenic abscesses were not obtained from any one source. These lesions were sent to the laboratory from Idaho, Illinois, New York, Oregon and Utah.

#### COMMENT

It is evident from a study of material secured from widely separated localities that the structural character of nodules situated in the subcutaneous tissues of cattle that react to tuberculin cannot be correlated with the geographic origin of the lesions. This would lead one strongly to suspect a causative factor common to all. Grossly, the lesions were without significant difference regardless of their origin, although Traum<sup>5</sup> mentioned the involvement of the regional lymphatic structures in certain cases in California. Apparently, so far as the histologic response is concerned, the so-called skin lesion of cattle is essentially the same throughout the United States. It is reasonable to assume that occasionally a nonspecific type of abscess may sometimes be confused at necropsy with the lesion that I have designated as tuberculous. Histologically, the distinction is not difficult.

In any review of the subcutaneous nodule, the etiology of these structures is usually considered. Although this study was confined, by nature of the material, to the morphologic aspects of the disease, speculations as to the agent responsible for inception of the changes cannot be avoided. Even though Traum, who first reported the condition in the United States in 1916, did not agree that the disease was tuberculous, the condition is considered by many investigators as representing a variety of tuberculosis. The evidence in support of the contention that the lesions are expressions of infection with *Mycobacterium tuberculosis* can be stated briefly as follows: (1) The histopathologic changes of the lesions are often indistinguishable from those induced by *Mycobacterium tuberculosis*; (2) acid-fast bacteria, morphologically not essentially unlike those responsible for true tuberculosis, occur in many of the lesions; (3) cattle affected with subcutaneous nodules frequently

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5. Traum, J.: *Cornell Veterinarian* **13**:240, 1923.

react to a diagnostic injection of tuberculin, and (4) cattle with these tubercle-like nodules are often from herds in which genuine tuberculosis has been demonstrated previously.

As impressive as this evidence may be, it is largely presumptive and does not constitute definite proof that *Mycobacterium tuberculosis* is the provocative agent. On the contrary, formidable arguments can be advanced to support the point of view that the infection is not truly tuberculous. These might be summarized briefly as follows: 1. The histologic aspects of a tubercle-like structure are unreliable criteria as to the nature of the etiologic agent responsible for a given lesion. The so-called tubercle is the result of a nonspecific, cellular reaction. Anatomic units or "tubercles" remarkably similar in cellular detail can be induced experimentally with such markedly dissimilar agents as *Mycobacterium tuberculosis*, *Brucella abortus* and silicon dioxide. Therefore, the histologic characteristics of a given lesion are no criteria of its specificity. 2. Attempts to culture from the lesions acid-fast bacteria that will cause tuberculosis in experimental animals have failed, as have attempts to produce tuberculosis by injection into cattle, swine, rabbits, guinea-pigs and chickens of emulsions of tissue prepared from the lesions.<sup>6</sup> 3. A positive reaction to mammalian or avian tuberculin does not indicate an infection with the organism of mammalian or avian tuberculosis respectively. Group reactions may occur in which animals sensitized by infection with the organism of human or bovine tuberculosis may react to avian tuberculin, and conversely, those infected with the organism of avian tuberculosis may react positively to mammalian tuberculin.<sup>7</sup> Sensitization to tuberculin may be due, in these cases, to undescribed and unidentified species of *Mycobacterium* in the lesions, or to a transitory residence in the animal of the bacilli of avian tuberculosis. 4. The localization of the lesions and the fact that in a large percentage of the affected animals internal lesions of tuberculosis are not discovered are contrary to what might be expected of the organism of true tuberculosis.<sup>7a</sup>

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6. In two instances I have succeeded in obtaining the organism of avian tuberculosis from subcutaneous lesions of cattle, but I do not believe this organism of significance in the pathogenesis of the condition; too many additional lesions have failed to yield such organisms.

7. Dolgopoi, Vera B.: *J. Infect. Dis.* **49**:216, 1931. Feldman, W. H.: *J. Am. Vet. M. A.* **83**:344, 1933. Schalk, A. F.: *ibid.* **72**:852, 1928.

7a. Further evidence attesting to the localized character of the disease was recently brought to my attention by Dr. C. U. Duckworth, of the California Department of Agriculture, Sacramento. In a personal communication, Dr. Duckworth presented evidence showing that sensitivity to tuberculin disappears following the surgical removal of tuberculoid lesions of cattle. The procedure was carried out among approximately 220 animals that were affected with lesions of this character, and when the animals were subsequently retested with tuberculin, they failed to give a positive reaction.



Although it can be stated that information available is definitely against the possibility of the tuberculoid subcutaneous nodule of cattle being due to a tuberculous infection, the final answer to the question of etiology must await additional investigation. The recent work of Daines and Austin,<sup>8</sup> in which a variety of heretofore undescribed acid-fast organisms was isolated from a large number of so-called skin lesions, may be significant. However, until the lesions can be reproduced experimentally more or less consistently by the organisms isolated from the spontaneous lesions, the part of these organisms in the pathogenesis of the lesions cannot be precisely stated.

Some, including Carpenter and Goldberg and Runnells, have made comparative studies of the so-called skin lesions of cattle and those of lupus vulgaris of man in an attempt to recognize a possible relationship between the two conditions. Carpenter and Goldberg considered the two diseases sufficiently alike structurally to conclude that the lesions in cattle "are identical with skin tuberculosis (lupus vulgaris) in man." Runnells also noted a morphologic similarity between the lesions of these two conditions, but pointed out some significant differences in that the lesions of lupus vulgaris are avascular, do not have a perivascular inception and appear to be more progressive.

I have also examined material from cases of lupus vulgaris in man, and although histologically a general resemblance to the subcutaneous lesions of cattle exists, the fallaciousness of drawing conclusions from analogous lesions in different species should be kept in mind. I have shown<sup>9</sup> in experimental tuberculous infections that the character of the reaction of the tissue to a given form of *Mycobacterium tuberculosis* varies with the species which has the infection. Tuberculosis is a disease of inconsistencies, and proper consideration must be given to many factors before the exact character of a given lesion can be ascertained. Therefore a morphologic comparison of the lesion of lupus vulgaris of human beings with the subcutaneous nodules of cattle for the purpose of determining a possible etiologic relationship is likely to lead to wrong conclusions.

That rarely lesions of the subcutaneous tissues may represent a true tuberculous infection is evident from the reports of Day,<sup>10</sup> Daines and Austin and Nieberle. Daines and Austin in one instance isolated *Mycobacterium tuberculosis* (bovis) from an animal which had internal lesions as well as cutaneous lesions of tuberculosis.

Nieberle<sup>11</sup> reviewed briefly a case originally described by Joest, in which there were multiple, flat, nodular masses in the subcutaneous tissues of the cervical, thoracic and abdominal regions of a heifer affected

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8. Daines, L. L., and Austin, Harold: *Am. Rev. Tuberc.* **27**:600, 1933.

9. Feldman, W. H.: *Arch. Path.* **11**:896, 1931.

10. Day, L. E.: *J. Am. Vet. M. A.* **72**:782, 1928.

11. Nieberle, K.: *Ergebn. d. allg. Path. u. path. Anat.* **25**:631, 1931.

with generalized tuberculosis. Nieberle also mentioned cases of tuberculosis of cattle in which the cutis was involved, and Besnoit and Robin<sup>12</sup> reported the occurrence of a similar case. The animal in Besnoit and Robin's case was an old cow with tuberculous lesions of the subcutaneous tissue on the right side of the wall of the thorax. At necropsy, generalized tuberculosis was observed, and the subcutaneous involvement was found to represent an extension from a subpleural tuberculous abscess. Lesions of the skin and subcutaneous tissues induced by the organism of bovine tuberculosis must occur rarely, and when present are probably, in the majority of instances, part of a tuberculous process present primarily elsewhere in the body. Such lesions must be assumed to arise by direct extension or as a result of a hematogenous infection. Differing in this regard are the often encountered subcutaneous tuberculoid lesions which are apparently not due to the organism of true tuberculosis and are limited in their distribution to the exterior surfaces of the body.

#### SUMMARY

A histologic study was made of the spontaneous tuberculoid lesion of the subcutaneous tissues of cattle in which the reaction to tuberculin had been positive and in which lesions of true tuberculosis were not found. The specimens were obtained from twenty-one states of the United States and from Canada. There were thirty-four specimens which were considered tuberculoid because of the resemblance of the histologic characteristics to those of lesions of tuberculosis. Fourteen other specimens disclosed lesions that were considered nonspecific pyogenic abscesses, and two others revealed only thickening of the corium; one was found to represent an adenoma of the sebaceous glands. Acid-fast organisms were present in twenty-two, or approximately 64 per cent, of the thirty-four lesions that were designated as tuberculoid.

No significant differences were observed in the character of the tuberculoid lesions in different sections of the United States, and the one lesion obtained from Canada was similar to the others studied.

#### CONCLUSIONS

The subcutaneous tuberculoid or so-called "skin lesion" of cattle which react positively to tuberculin appears to be widely distributed over the United States. The literature at this time suggests that the condition occurs rarely in Continental Europe or in the British Isles.

Histologically, the lesion is essentially a monocytic and epithelioid cellular reaction, with a peripheral and granulomatous encapsulation.

Although the lesions usually closely resemble in appearance those of tuberculosis, evidence at this time indicates that agents other than the bacteria of true tuberculosis are probably responsible for the disease.

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12. Besnoit, C., and Robin, V.: *Rev. vét.* 5:409, 1923.

# General Review

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## THE NATURE OF SO-CALLED XANTHOMA

A CRITICAL REVIEW

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AND

M. G. SEELIG, M.D.

ST. LOUIS

### INTRODUCTION

Certain granulomatous or tumor-like formations encountered in the most diverse parts of the body and under a great variety of clinical conditions are distinguished by a special cell form, the main characteristics of which are a comparatively large body of protoplasm with distinct multiangular borders and a foamy structure, the latter brought about by the removal of numerous droplets of lipoids by the fat-dissolving solutions employed in the routine methods of preparation.

Since such cells were first observed in a peculiar tumor of the skin named "xanthoma" or "xanthelasma" (on account of its yellow or yellowish-brown color), they are called "xanthoma cells" or, more descriptively, "foam cells."

Subsequent observations have established a number of facts which, in their potential correlation, have made the pathology of the xanthoma cell highly interesting. These are: 1. Pathologic structures containing xanthoma cells may occur in many other organs besides the skin. 2. Xanthomatous formations occur as solitary, but also as multiple, sometimes widely distributed tumors. 3. Xanthomas, both single and multiple, are often encountered in patients with an increase of cholesterol in the blood. 4. The droplets that give the xanthoma cell its characteristic appearance consist of lipoids, chiefly cholesterol and its esters.

The occurrence of xanthomatous tissue changes simultaneously with a disturbance of the blood chemistry suggests that these formations are not neoplasms but products of abnormal metabolism. Many authors assume them to be storage tumors produced by the pathologic fixation of superabundant lipoids resorbed from the circulation, and explain their structure more on the lines of a granuloma than on those of a neoplastic cell proliferation. It has been proposed to discard the term "xanthoma"

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because these lesions are not true autonomous growths and at best deserve the ending "oma" to express a tissue reaction analogous to that following an injection of paraffin, namely, the so-called paraffinoma. With such philologic criticism we are in accord, and this study has been prompted by the desire for a clearer etiologic understanding of the pathology of the foam cell, which would permit a scientific classification of its various forms.

Rowland, in his meritorious treatise on the anomalies of lipid metabolism, reviewed all detailed knowledge pertaining to this problem and voiced the opinion that all tumors or granulomas containing xanthoma cells are manifestations of one disease, a disturbance of the lipid metabolism to which he gives the name "xanthomatosis."

Rowland also advocated a monistic conception of the histogenesis of the foam cells. According to him and others, foam cells are always cells of the reticulo-endothelial system, which acquire their shape as the result of their phagocytic reaction to the presence of lipoids (increase in normal lipoids or the presence of foreign lipoids). This contention is not merely of theoretical importance. The surgical pathologist frequently encounters biopsy specimens in which the predominant finding is xanthoma cells, usually in connection with a varying degree of fibrous tissue formation. The question arises whether it is justifiable to diagnose xanthomatosis in the sense of Rowland's conception on the basis of this microscopic finding alone, or whether pathologic formations of different and entirely unrelated genesis can assume the xanthomatous structure. It is evident that if Rowland's theory is applicable to all cases, the problem of xanthomatous formation is settled in very simple fashion and the pathologist is justified in making the microscopic diagnosis of xanthomatosis in every instance of foam cell tumors.

According to recently accumulated observations, the pathology of xanthoma is of very wide scope. Innocuous and rather common lesions like the xanthoma of the eyelid or the so-called giant cell sarcoma of the tendon sheath line up with such formidable systemic disorders as Niemann-Pick's disease and Gaucher's splenomegaly. Rowland's classification may be given in order to illustrate how the chapter on xanthoma has expanded:

- A. Essential or primary disturbances of lipid metabolism with characteristic clinical and anatomic expressions
  1. Essential xanthomatosis (the variety affecting the skin, mucous membrane, tendon sheaths or viscera)
  2. Niemann-Pick disease
  3. Schüller-Christian disease
  4. Gaucher's disease
- B. Symptomatic or secondary forms of lipid metabolism
 

Such forms are associated with diabetic lipemia and certain disorders of the liver and kidney in which the metabolic disturbance is secondary

A causal relationship between the formation of xanthomas and the abnormal cholesterol content of the blood is most convincingly demonstrated in certain cases of diabetes. Here, disappearance of the tumors (or granulomas) follows restoration of the blood chemistry to normal conditions. In other forms of xanthomatosis the involvement of the reticulo-endothelial system is most obvious, as in Gaucher's disease, Niemann-Pick's disease and other generalized forms with extensive involvement of the bone marrow.

The monistic conception of xanthomatous granulomas or tumors has strong arguments in its favor, but from our own experience and from a perusal of the literature we have gained the impression that for many cases, especially for solitary tumors, this explanation is without sufficient proof.

It seems to us that the time is ripe for a critical analysis of what we have called the "monistic" or, possibly better, the "unifying" theory of xanthoma formation. As this theory is based on two assumptions, (1) the derivation of the xanthoma cell from the elements of the reticulo-endothelial system and (2) a preceding hypercholesteremia, we shall review the subject under the two heads of pathologic anatomy and pathologic physiology. This separation is advisable because the correctness of one phase of this theory may stand in spite of an apparent weakness of the other.

#### I. PATHOLOGIC ANATOMY

In his textbook, "*Traité des maladies de la peau*," published in 1836, Rayer portrayed, under the title "*plaques jaunâtres*," yellowish skin efflorescences, usually located on the eyelids, but occasionally found in other parts of the integument. Several Englishmen, Addison and Gull, Wilson and W. F. Smith, published the first detailed clinical reports. The last mentioned author used the term "xanthoma," which soon became the accepted term for this lesion. Microscopic examinations were reported by Pavy, Fagge, Hutchinson, Waldeyer, Virchow, Kaposi and Chambard, all of whom are quite in accord in describing the histologic picture. They found a proliferation of the cellular and fibrous elements of the cutis and peculiar large cells loaded with numerous fat droplets. While most of these authors considered the whole process, including the appearance of the cells containing the fat droplets, to be a reaction of the connective tissue, other contemporary students, e. g., Wilson, Hebra<sup>1</sup> and Kaposi, Geber and Simon, interpreted the fatty cells as hypertrophic formations of sebaceous glands to which, indeed, they have a striking resemblance in view of the foamy structure of their protoplasm.

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1. Later on Hebra revised his opinion; he regarded xanthoma as a peculiar fatty neoplasm.

Chambard published excellent lithographs of microscopic sections and adopted the name "xanthoma" from W. F. Smith for the pathologic findings, thus introducing this term into the continental literature. Among the great number of older publications on the histology of xanthoma, the ones by Török and Geyer seem to be of special interest. Török found the xanthoma cells arising from the adventitia of small vessels, but, not knowing that their granules differ chemically from the fat in regular adipose tissue, he believed them to be embryonal fat cells. Geyer, on the other hand, brought forth equally good evidence for the origin of these cells from the intima of the smallest lymph vessels.

Toepfer described a xanthoma with absence of foam cells, the fatty granules being contained in the typical spindle cells of hypertrophied connective tissue.

The early investigators also discussed the general pathology of these lesions. Waldeyer (1871) emphasized the difference between a fatty cellular degeneration and the infiltration of cells with fat in his case of xanthelasma palpebrarum: "The fat droplets assume considerably larger size and there is no evidence that these fat inclusions damage the cells, because, after extraction of the fat, a good deal of protoplasm becomes visible, and a well formed nucleus appears. No colliquation of cells or any detritus can be found."

Virchow corroborated Waldeyer's description with the report of a case of molluscum lipomatodes (in his terminology) and a xanthomatous growth of the conjunctiva which had obliterated the cornea. He described a xanthoma nodule as follows: ". . . The peripheral and the young elements consist almost entirely of proliferating connective tissue cells, which occasionally somewhat resemble sarcoma on account of their great number and their size. Nowhere was there any evidence that the progressive infiltration with fat initiated a necrobiotic process. This process may be compared with the fatty metamorphosis of certain connective tissue structures as, for instance, that of the inner lining of the aorta, but the integrity of the cells (in the case of xanthoma) is not interfered with. . . ." This reference to the atheromatous degeneration of the aorta anticipated the establishment, at a much later date, of a chemical relationship between these processes.

Emphasizing the rôle of the peculiar foamy cells in the histologic picture of xanthoma, de Vincentiis and Touton regarded these lesions as neoplasms, inferring that every xanthoma cell is derived directly from another one of the same structure. Unna was very specific about a totally different histogenesis. In a cutaneous xanthomatosis in a diabetic patient, he found fibroblasts in all stages of transition to foam cells. In the center of a xanthoma, surrounding fat crystals, were enlarged epithelioid-like cells stuffed with fat droplets and not infrequently rup-

tured by these enclosures. Toward the periphery, the cells gradually assumed the shape and size of regular fibroblasts while the content of fat droplets decreased conspicuously. A zone of normal spindle cells formed the border of the focus. Krzyształowicz substantiated these observations and concurred with Unna in his opinion about the fibroblastic origin of the xanthoma cells.

By this time the clinical aspects of xanthomatous lesions had become quite complex. It was known that xanthomatous lesions of the skin were frequently associated with jaundice and diabetes. They were observed as solitary tumors, as symmetrical lesions (i. e., on the eyelids) and as multiple and even generalized growths. Macroscopically, the tumors considered to belong to this group varied greatly in size, site, depth, color and consistency. The question arose whether these different pathologic formations could still be considered to express an entity on the strength of their histologic appearances.

Török found typical foam or xanthoma cells in all the different clinical forms (xanthoma of the eyelids, the single and multiple tumors of the skin and the xanthomatous lesions associated with jaundice). However, he regarded the diabetic form as a separate disease of an inflammatory nature in which an infiltration with exudate cells and a necrosis of fatty degenerated cells prevail.

Unna, on the contrary, considered the diabetic xanthoma typical of and identical with generalized xanthoma. But his comparative studies led him to the conclusion that the solitary xanthomatous tumors containing giant cells should be separated from the groups of generalized xanthoma; that, likewise, xanthoma palpebrarum is a pathologic process *sui generis* because the accumulation of fat takes place extracellularly in the lymph spaces.

If one accepts these somewhat divergent observations of the earlier students as authentic it appears that cutaneous xanthoma shows as much variation in histologic detail as in clinical appearance. There is, however, no histologic formation specific for any one of its clinical forms in spite of repeated attempts to reserve certain microscopic peculiarities for some of them. Any such contention advanced by an author has been disproved by an equally competent observer of clinically identical material. The differences in the microscopic observations are apparently incidental to the individual cases and more dependent on the developmental stage of the lesion than on any etiologic specificity.

More recent observations on a larger series of cases of cutaneous xanthoma support the conclusion that variations in the histologic structure are without significance for an etiologic differentiation.

Additional evidence for a close relationship of all the different clinical and histologic pictures of cutaneous xanthoma was gained when

Stoerck discovered that the characteristic cellular inclusions of the xanthoma cell were not fat but a doubly refractive lipid substance. Although Stoerck believed that this lipid was a product of protein decomposition (he called it "protagon"). Pinkus and Pick soon identified it as a cholesterol compound and associated it with the hypercholesteremia known to exist often in patients suffering from generalized xanthomatosis. The same chemical substance was found in all forms of xanthoma—xanthoma of the eyelids, solitary xanthomatous tumors and the xanthoma cells that sometimes appear in the vicinity of chronic foci of suppuration. The details of the histologic structure came to be considered as of less importance than the presence of the cholesterol compound in the tissue. Lubarsch and Bross studied a case of severe and rapidly fatal diabetes with a yellow discoloration of the total skin surface, believed to be jaundice, in which an autopsy disclosed extensive xanthomatosis (cholesterol ester deposits) in the visceral organs and the bone marrow. Under the microscope, the lipid droplets were found: (1) as extracellular masses engorging lymph spaces and small vessels; (2) within the endothelial cells of lymphatic and blood capillaries; (3) in typical connective tissue cells and in proliferating spindle cells arranged along the course of blood vessels; (4) in xanthoma cells which appeared to develop from the lining cells of the lymph spaces. The yellow discoloration of the skin was due to a heavy infiltration of the cutaneous lymph spaces with columns and droplets of lipid substance.<sup>2</sup> Lubarsch concluded that the histologic details of xanthomatous lesions are of only minor importance. The essential pathologic factor is the flooding of the circulation with lipoids and their stagnation in the lymphatic pathways. Incidentally, and possibly owing to mechanical circumstances, endothelial or connective tissue cells may react to the increasing concentration of these substances with the formation of foam cells. Gans, in his recent textbook on the histology of cutaneous diseases, also doubted the significance of histologic differences in the structure of xanthomas. He expressed the opinion that the cells which participate in the formation of xanthoma or foam cells are either endothelial or connective tissue cells, and that the infiltration with fatty acid esters of cholesterol is a phenomenon of storage rather than a degenerative process of the protoplasm, because karyokinesis takes place in young xanthoma cells. In older foci, a replacement of the lipid-containing cells by dense connective tissue takes place; this replacement may go so far as to obliterate all traces of xanthomatous substance and

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2. Diabetic xanthosis has been observed and described by von Noorden and Umber without reference to xanthomatosis and hypercholesteremia. According to recent investigations, the discoloration of the skin is usually due to an excess of lipochromic substances in the blood (Burger and Reinhart).



cells, the only remnant of the previous cholesterol infiltration being a yellow pigment, lipofuscin, in cells reduced to normal dimension and shape.

Pollitzer and Wile, describing the histologic variability of cutaneous xanthomas in one and the same case, claimed that the xanthoma of the eyelids is histologically different from other forms of cutaneous xanthoma. They found that the condition is due to a degeneration of the muscle fibers contained in the eyelids.

*Visceral Xanthomatosis.*—Visceral xanthomatosis associated with the cutaneous lesions was described by the early English observers. In the more recent literature, Murchison is often cited as having published the first report, although in his paper dealing with a case of jaundice and xanthoma of the eyelids he mentioned only a cirrhotic change in the liver.

Fagge reported the case of a patient who had intensive jaundice of many years' duration, xanthomas of the eyelids and cutaneous tumors on the hands. At autopsy, cirrhosis of the liver was found as the cause of the jaundice. The vocal cords and the mucosa of the trachea<sup>3</sup> were the site of yellow patches with a histologic structure identical with the xanthomatous lesions of the skin. Furthermore, similar patches occurred on the endocardium and on the intima of the large vessels including the pulmonary artery. Xanthomatous tumors were also found arising from the tendon sheaths of the fingers.

Pye-Smith had a patient suffering from intermittent jaundice due to stones in the common bile duct who, at a later stage of the jaundice, showed xanthomatosis that was at first confined to the eyelids and palmar surfaces of the hands. The cutaneous lesions spread slowly, uninfluenced by temporary improvement of the jaundice. She finally succumbed to erysipelas. The autopsy confirmed the diagnosis of stones in the common bile duct. Patches of xanthoma were found on the peritoneal surface of the spleen and in the mucosa of the bile ducts.

In a patient with generalized cutaneous xanthoma, Balzer found patches on the endocardium and pericardium. The cause of death was an extensive pulmonary tuberculosis.

Interesting is the publication of Lehzen and Knauss concerning the occurrence of generalized cutaneous xanthomatosis in two sisters, 11 and 9 years of age. The elder sister died, probably from heart failure. The clinician (Leube) had suggested the possibility of xanthomatous lesions in the heart. At autopsy, this suspicion was confirmed. The mitral valve carried xanthomatous plaques, likewise the intima of the aorta, pulmonary artery and coronary vessels. The left carotid artery was almost completely stenosed by the xanthomatous proliferations, xanthoma cells being present in the walls of the vessels. Multiple xanthoma was also found on tendons and joint capsules of the hands.

Pinkus and Pick reported on pachymeningitis interna xanthomatosa in a patient with hypertrophic cirrhosis of the liver, with jaundice and extensive xanthomatosis of the skin.

Low's case is almost identical with the one described by Lehzen and Knauss.

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3. Observations on xanthomatosis with a localization in the larynx and trachea were recently published by Finney, Montgomery and New.

Chvostek's publication is especially valuable on account of a detailed histologic study. The autopsy of a jaundiced patient revealed a diffuse proliferation of what appeared to be typical xanthoma cells in the liver. These cells were seen developing from Kupffer's cells and producing a distortion of the hepatic cell columns and biliary capillaries. In the skin of the same patient there existed a rather generalized xanthomatous eruption, which on microscopic examination was found to consist of foam cells. These cells, however, appeared to be transformed fibroblasts, no connection with endothelial cells being demonstrable. Still more remarkable were the histochemical differences in the two types of foam cells in the same patient. The cellular inclusions of the foam cells in the cutaneous lesions were doubly refractive in polarized light and stained with lipoidophil stains; these reactions could not be reproduced in the foam cells of the liver. Chvostek considered it possible that unknown chemical reactions in the liver interfered with the typical reactivity of the lipoids. Indeed, the histochemical methods for the detection of the so-called lipoids are based on such very complex and incompletely understood reactions as to make the evaluation and interpretation of sections so processed rather difficult (Arndt). Chvostek's case also suggests that hypertrophic biliary cirrhosis might be produced by a xanthomatous proliferation of the endothelial cells of the liver. Similar processes might have been responsible for some of the other cases of xanthomatosis associated with biliary cirrhosis and jaundice, this histologic process often having been obscured by the fibrous end-stage. Hardaway and Hallopeau independently advanced the theory that localizations of xanthoma in the liver and pancreas, respectively, produce the jaundice and the diabetes so frequently found associated with xanthomatous conditions of the skin. Of course, these hypotheses cannot be regarded as a generally valid theory. Sickemeier described a case of cutaneous xanthomatosis with cirrhosis of the liver and jaundice, in which the process in the liver was due to a miliary hepatic tuberculosis. Schulte, also, reported a case of visceral xanthomatosis, characterized by severe jaundice of long duration, due to an obstruction of the common bile duct by a carcinoma of the pancreas. Foam cell proliferations were found in the capsule of the liver and spleen and in a solitary xanthomatous tumor of the skin in the bend of the elbow.

Lubarsch's case (see also Bross), referred to before, also represents typical visceral xanthomatosis.

We have reviewed these reports somewhat in detail, because they show convincingly not only that xanthomatous cell proliferations may occur in internal organs, but also that the cutaneous and visceral lesions are etiologically related. This does not obviate certain histologic and sometimes even histochemical differences in the various manifestations in one and the same case.

*Xanthomatosis of the Hematopoietic Organs.*—Quite recently these pathologic conditions have become the object of numerous investigations. Unlike the clinical forms enumerated in the foregoing section, this group of xanthomatous diseases shows a lesser tendency to be manifested in lesions of the skin. This fact may explain why clinical recognition has been achieved only in recent years. Indeed, in many instances the xanthomatous cell proliferation is restricted to the organs of the hematopoietic system.<sup>4</sup>

A diabetic metabolism occasionally may here, as in other forms of xanthomatosis, account for the development of the lesions. Representative of such a secondary xanthomatosis in the hematopoietic organs is the so-called diabetic lipoid hyperplasia of the spleen. (The literature has been reviewed by Pick.) This condition develops occasionally in patients who succumb to a severe, rapidly progressing diabetes, with lipemia and hypercholesteremia. The typical pathologic changes consist in a replacement of the splenic pulp by foam cells containing droplets of cholesterol esters. In a few cases the same changes have been found simultaneously in the bone marrow, in visceral lymph nodes and in the liver. In cases in which the xanthomatous process involved also the intima of large vessels and the endocardium, the pathologic picture approached that of a type of generalized diabetic xanthomatosis described by Lubarsch and Bross.

The xanthomatosis of hematopoietic organs *without* underlying diabetic diathesis is, in the light of present day knowledge, not an entity but a group of several interesting diseases distinguished from one another by clinical and chemical peculiarities. Three typical forms are recognized: (1) Gaucher's disease; (2) the Hand-Christian-Schüller syndrome, and (3) Niemann-Pick's disease (Rowland; Chiari; Chester; Henschen).

The histology of Gaucher's disease differs noticeably from that of the other forms, because the large pale cells found in this condition have not the foamy protoplasmic structure of xanthoma cells. However, it is included in this group for the reason that these cells are of the same derivation as the foam cells and also contain a lipoid substance.

The Hand-Christian-Schüller syndrome is produced by xanthomatous changes of the bone marrow of the skull. By the pressure exerted on the adjacent organs the symptoms of exophthalmos, diabetes insipidus and possibly other pituitary dysfunctions arise. The xanthomatous proliferations sometimes involute spontaneously and, according to Sosman and Lazarewa, are sometimes radiosensitive. Abrikossoff and

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4. Peripheral lymph nodes which might present themselves for biopsy are rarely involved. The case reported by Robertson and Warren seems to be exceptional in this respect.

Herzenberg (1929), Herzenberg (1928), Pick and Lubarsch have discussed the question whether cases without the typical distribution of foci in the cranial bone marrow, as described by the original observers, may justly be included in this group. Chiari and Davison have made a special study of xanthomatous lesions in the brain in this disease. The pituitary disturbances are frequently the result of a xanthomatous involvement of the nerve or glandular tissue and not always due to pressure from adjacent xanthomatous lesions of the bone. Occasionally, xanthomatous (foam cell) foci can be found widely distributed throughout the central nervous system.

Niemann-Pick's disease is considered to be a racial and familial disease occurring in female infants of Jewish extraction. It usually terminates fatally before the patient reaches the second year of age. Xanthomatous proliferations are found mainly in the spleen and liver, but may involve almost any organ—bone marrow, lymph glands, lungs and brain.<sup>5</sup>

The literature on these diseases is almost contemporary and is still increasing at a fast rate. Adequate surveys have been made repeatedly (Pick; Rowland), and so it may suffice to point out certain histologic details. All three diseases are characterized by the proliferation of large pale cells, the histology of which shows certain special features. The Gaucher cell does not assume the honeycombed or foamy appearance, because the specific lipid substance, kersin (Epstein), does not dissolve in fat solvents as do the lipoids in other xanthoma cells, nor does it give color reactions with fat stains or double refraction in polarized light. Certain fibrillar structures can be produced in its protoplasm with special staining methods, which help to identify the cell. Gaucher cells develop in the spleen from reticulum cells, while the endothelium of the venous sinus remains at rest. A second source of the Gaucher cells is often the periadventitial cells of the splenic arterioles. Gaucher cells may vary in size and shape considerably more than other xanthoma cells. They may be found also in the liver and bone marrow; in a few cases the latter has been found to be predominately involved.

The foam cells found in Hand-Christian-Schüller's disease are of typical shape and contain droplets of cholesterol and its fatty acid esters. According to Pick, they originate from reticulum and periadventitial cells exclusively. A slowly fibrosing granulation tissue supervenes and replaces the foam cells in a later stage of the process. This granulation tissue is entirely absent in the histologic picture of Gaucher's disease and Niemann-Pick's disease, in which fibrosis occurs, too, but is brought about by a direct condensation of intercellular substance.

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5. Pick and Bielschowsky believe that congenital amaurotic idiocy, type Tay-Sachs, is a lipoidosis with identical histology distinguished from Niemann-Pick's disease by the predominantly cerebral localization (Pick).

The foam cells found in Niemann-Pick's splenohepatomegaly have also the appearance of typical xanthoma cells. Their cellular inclusions react positively to lipid stains, although with a different shade of color. Chemically, they have been identified as phosphatid lipoids or lecithin. The pathologic process is far more generalized than might be suspected from the appearance in the gross. In addition to the spleen and liver, the visceral lymph glands, bone marrow, lungs, thyroid gland, salivary glands, the connective tissue in general, the muscle tissue (especially that of the heart) and frequently the central nervous system show phosphatid substances deposited either in regular tissue or in foam cells. The foam cells develop mostly from reticulum cells and to a lesser degree from the endothelial lining of small vessels. However, commensurate with the diffuseness of the process, other cell types undergo foamy transformation—liver cells, the cells of the pulmonary alveolae, fibroblasts, glandular epithelium, muscle and nerve cells.

These three diseases form the group of primary xanthomatosis of the hematopoietic system. Each disease is clinically, histologically and chemically sufficiently different for one to ascribe autonomy to each of them. Considerable variations in the clinical course and the anatomic distribution may be found in the individual cases, making an even approximately complete description of all known varieties and possibilities an extensive study. Evidently the study of these diseases has furnished much support to the theory that foam cells in general are cells of the reticulo-endothelial system in a stage of reaction to lipid substances. On considerably less convincing evidence, the observations on cutaneous and visceral xanthomatosis may still be considered compatible with this conception.

The validity of this theory appears to be less well established in the groups of lesions which we shall now take up, even though it is difficult to doubt their systemic nature.

*Xanthomatosis of Tendons and Paratendinous Tissue.*—The case of Fagge, Lehzen and Knauss, Low, and Hardaway, already cited, show that tumors involving the tendons and paratendinous tissue are one of the potential manifestations of xanthomatosis. Reports of similar cases have been repeatedly published:

Poensgen observed two young boys, cousins, with numerous xanthomas of the skin, tendons and paratendinous tissue without underlying diabetes or icterus.

Carry reported a similar case occurring in a 10 year old girl in whom, in addition to xanthomatous efflorescences of the skin, subcutaneous tumors of the same type arose from the tendo achillis, the patellar ligament and the periosteum of the malleoli. In 1889, Ehrmann (Török) reported on the brother of one of Poensgen's patients, afflicted with xanthomatous tumors of a similar distribution and a probable visceral involvement as suggested by the development of ascites and the enlargement of abdominal veins.

Addison and Gull, pioneer investigators of xanthomatosis, mentioned as early as 1851 the involvement of tendons of the fingers in one of their first three cases. Török's collective paper, published in 1893, refers to seventy cases of xanthoma in ten of which tendinous and paratendinous tumors were mentioned. The series of comparable cases was continued in 1910 by Arning with a report of familial xanthomatosis afflicting a mother and four of her nine children. Other contributions have been made by Hoessli, Knowles and Fischer, Wolodin, Chalатов, Krogus, Harbitz, McWorther and Weeks, and Weidman and Freeman. Chalатов found the xanthoma cells developing from connective tissue cells (cited by von Albertini). Reports have been published more recently by Kusnetzowsky, Krecke, von Albertini, Ollerenshaw, and Nathan and Herrmann.

Tendons and tendon sheaths evidently become rather frequently involved by xanthomatous tissue changes. The intimate relation between these multiple tendon tumors and other forms of xanthomatosis is evidenced by many clinical features in the individual cases. Why a systemic xanthomatosis may manifest itself in these structures, however, can hardly be explained by the theory that it represents a reaction of reticulo-endothelial cells. Aschoff and Landau's definition of this cell system does not include any cell complexes located in these tissues. While we admit the systemic nature of this type of tendinous xanthomatosis, we shall presently refer to a group of xanthomatous lesions of identical anatomic location in which the diathetic character of the xanthomatous process is debatable.

*Solitary Xanthomatous Giant Cell Tumor.*—Arising in the same tissues, from tendons, paratendinous tissue, aponeurosis or other dense connective tissues solitary tumors have been described by Chaissagnac (1852), Spencer-Wells (1857) and later by Billroth and Czerny (1869) and subsequently in literature quite remote from the sphere of interest of the students of xanthoma (for the literature, see von Albertini).

This solitary, well encapsulated and, in most cases, definitely lobulated tumor is found usually on the hand or the foot in intimate connection with a tendon but rarely involving it. The histologic structure resembles that of a sarcoma of the epulis type, the constituent cells representing several types of mesenchymal cells. It contains spindle or round cells, giant cells, connective tissue fibers and, as a rule, a brown pigment, hemosiderin. Reverdin emphasized the benign clinical course. Heurteaux described the giant cells as being identical with the myeloplax cells of the bone marrow, and Dor found xanthoma cells in this tumor. Dor therefore contended that tumors of this type, heretofore called *myelomes des gaines tendineuses* by the French and *xanthosarcoma giganteo cellulare pigmentosum* by the Germans, belong to the category of xanthoma. Pathologists still have not come to an agreement on this question. In connection with our problem, the uniform genesis of foam cell tumors, this controversy is of definite importance.

At present two opinions concerning their nature are generally voiced:

- (1) These tumors are benign neoplasms of the connective tissue with a more or less incidental admixture of xanthoma cells, these cells being rather unessential or even inconstant elements of the new growth (Spiess; Landois and Reid; Katsurashima; Krogius; von Albertini).
- (2) These tumors are granulomas following traumas of the tissues or arising from humoral disturbances or caused by both (Fleissig; Weil; Seyler; Sprenger).

After comparing the more recent descriptions, and after studying our own cases, we have the impression that in their microscopic details they do not differ essentially from other xanthomatous formations. The proliferation of fibroblasts, the giant cells, the hemosiderin pigment and the occasional infiltration by inflammatory cells are also found in lesions the non-neoplastic nature of which is well established. Thus the histologic analysis does not contribute much toward the solution of the question as to whether these tumors are neoplastic or identical with xanthomas in general. Von Albertini and Tramonto and Fittipaldi wished to include these tumors in the general category of mesenchymal giant cell neoplasms, more commonly found arising from periosteum, bones, joints, tendon sheaths and skin. They supposedly form an intermediate group between the fibromas and the sarcomas, and develop from embryonic mesenchymal rests. Xanthomatous features are as a rule found only in those tumors which are located at the tendons or in the skin. This is presumably due to chemical peculiarities of these tissues, which is also suggested by the predilection with which general xanthomatosis manifests itself in the same tissues. We have to refer to von Albertini's excellent presentation of this subject containing an extensive discussion of respective cases.

There are certainly features which suggest that these formations are true neoplasms. For instance, their well encapsulated and lobulated form is repeated by other tumors occurring in the same tissues, like chondromas and myxomas. In a considerable proportion of the giant cell tumors of these structures xanthoma cells have been found present only in small numbers or even totally absent (Coenen; Landois and Reid). A few of these tumors have shown tendencies to infiltrate, with erosion of joints and bones. Tumors of the bone marrow similarly composed and apparently related to the so-called osteitis fibrosa cystica of von Recklinghausen<sup>6</sup> have been repeatedly found to contain foam cells (Kolodny; Krogius; von Albertini; Saralegui and Gonzales; Schröder; Lazarewa; Zeyland and Dega).

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6. Curiously enough, osteitis fibrosa cystica presents similar problems for an intelligent classification. The histologic and radiologic features characteristic of this condition are found in a localized and in a multiple bone disease. For the latter, in quite a number of cases, a hormonal-metabolic disturbance could be found as conducive to the osteitic changes (Mandl and others).

Investigators who approached the subject from the field of generalized xanthoma also have often proposed a dualistic classification of xanthomas. For instance, Unna separated xanthomatous tumors containing giant cells from the group of ordinary xanthomas, and Pinkus and Pick, who contributed so much to the theory of cholesterol diathesis, also believed that some of the xanthomas are neoplasms with a secondary admixture of xanthoma cells (see later paragraphs). Aschoff's school sponsors a classification of xanthomas which distinguishes between primary xanthomas, or tumors due to a hematogenous cholesterosis, and secondary xanthomas, or degenerative processes in tumors or inflammatory processes (Kammer).

*Xanthomatous Formations the Structure of Which Is Predominantly Neoplastic.*—In the following a survey is given of xanthomatous formations with a histologic structure dissimilar from that of typical xanthoma inasmuch as the cellular elements predominant in them characterize them as neoplasms.

**Endotheliomas:** The authority of Pick is again invoked for a number of cases reported by C. H. Smith:

Case 1. Tumor of the tongue with foam cells forming syncytia encroaching on normal-appearing muscle fibers.

Case 2. Tumor of similar composition removed from the labium majus pudendi.

Case 3. Foam cell tumor of the parotid gland.

Case 4. Psammoma of the dura, with xanthoma cells distributed among the spindle cells.

Pick and C. H. Smith interpreted these tumors as interfascicular lymphangio-endotheliomas with storage of cholesterol and subsequent transformation of their cells into foam cells, the storing being due to an abnormal metabolism of the tumor cells. A similar storage occurs in certain other tumors, which accumulate glycogen, although the parent tissue lacks this faculty.

Two cases of cystic lymphangioma, identical in their clinical aspects, are described by Kirch. In both instances, the tumor was removed from the subcutaneous tissue in the region of the knee. The cystic cavities contained cholesterol crystals suspended in a thin fluid. The walls of these cysts had the multicolored appearance of xanthofibrosarcoma (solitary xanthoma). Microscopically, the tumors were composed of two different elements: The first was endothelial cells in solid aggregates or forming cysts of greatly varying size. These cells contained hemosiderin and lipoid droplets and were found in all stages of transition toward large xanthoma cells. The second structural element was a sarcomatous stroma of the epulis type entirely free from demonstrable cholesterol infiltration and its cells showed no connections with the foam cells.

A third case of Kirch's was that of an endothelioma of the dura, the neoplastic character of which was especially ascertained by the occurrence of cells of the neurinoma type.

Beitzke found foam cells containing cholesterol esters in a tumor of the cerebellopontile angle of the ganglioneuroma type.



**Lipomas and Fatty Tissue:** Ewing mentioned the more or less frequent occurrence of lipoid-containing foam cells in lipomas of varying degrees of cell differentiation. More frequently similar appearing polyhedral cells are found which contain droplets of neutral fat and are arranged along the large vessels traversing the tumor.

Adair, Pack and Farrior described a tumor, 13 pounds (5.8 Kg.) in weight, removed from the anterior aspect of the thigh. According to the microscopic examination, this growth was a xanthomyxolipoma with foam cells predominant. A year later a recurrence showed the more uniform structure of liposarcoma. No information is given about the chemical nature of the intracellular droplets.

Proescher and Meredith described as multiple myxocholesterolipoma tumors occurring in a woman 32 years of age. Three of them were located subcutaneously and two intra-abdominally in the retroperitoneal space at the insertion of the mesocolon transversum and descendens, respectively. One of the abdominal tumors was removed, but a year after the operation the patient succumbed to a recurrence, with dissemination of tumor masses over the peritoneal surfaces, accompanied by ascites. These tumors consisted of myxomatous tissue with islands of foam cells giving the histochemical reactions of lipoids and showing double refraction in polarized light.

Xanthomatous transformation of the mesentery is rare although typical enough to be the subject of several publications. The xanthomatous changes take place mainly in the areolar tissue of the radix mesenterii (Schlagenhauser; Versé; Hirsch; Karoliny; Petri). Versé found in most of his cases a blocking of the lymphatic vessels due to tuberculosis, tumor of the liver or carcinomatosis of the thoracic duct or to other mechanical factors. Karoliny observed ten cases of mesenteric xanthomatosis, most of them occurring in elderly and overnourished patients. He considered the mechanical explanation advanced by Versé as not adequate, because he failed to reproduce experimentally xanthomatous changes in the mesentery by compressing or ligating the thoracic duct. Nevertheless, he confirmed Versé's and Hirsch's observation that the lymph nodes in the vicinity of the xanthomatous changes are regularly fibrosed. Petri refrained from offering an etiologic explanation of her case, which was that of a diabetic patient, 69 years old, who died from a far advanced carcinoma of the vulva.

**Epithelial Tumors:** Corten described a tumor, the size of a fist, removed from the subcutaneous tissue of the buttocks. The microscopic examination showed a stroma and a parenchyma in clear differentiation. The latter consisted of foam cells which in the larger aggregates formed pearl-like bodies giving a modified keratin reaction. The stroma consisted of a tissue of the type of giant cell sarcoma with myeloplax and foamy giant cells (Touton cells). The occurrence of the pearl-like bodies surrounded by foam cells suggested an epithelial derivation of these cells, and the tumor was assumed to have arisen from the misplaced anlage of a sebaceous gland.

Kneringer and Priesel examined a thymoma occurring in an elderly patient. Both the epithelial and the lymphatic elements were in a stage of neoplastic proliferation. Foamy protoplasmic structures were found in cells of both types. The thymus during its physiologic activity is one of the organs that contain greater amounts of cholesterol (Dietrich and Kleeberg). Possibly the chemical affinity of the parent tissue was responsible for the lipoidophil behavior of the tumor cells.

This explanation was also suggested by Corten for the foam cells of a metastatic growth in a suprarenal gland secondary to a carcinoma of the breast. Here the cancer cells contained more and larger droplets of cholesterol esters than the surrounding suprarenal cells. The primary tumor and the metastases in other organs lacked the lipid infiltration.

Kinoshita found foam cell structure and lipid infiltration in the cells of a carcinoma of the prostatic gland of otherwise typical appearance. Lipoids are not uncommonly found in high concentrations in normal prostatic glands.

Petri found that the cells in an adenocarcinoma of the stomach and its metastases in the liver assumed the appearance of typical foam cells. Examination in polarized light and differential staining with lipoidophil dyes, however, proved that the cellular inclusions were neutral fats and not cholesterol compounds. In accord with Pick's opinion, referred to before, this fat infiltration was interpreted as due to a peculiarity in the cell metabolism of this special neoplasm.

Foam cell structures in tumors of epithelial or mesothelial derivation are found, furthermore, in certain types of so-called hypernephromas. According to Ewing, certain hypernephroid tumors of the kidneys, which really develop from suprarenal rests, may be composed of lipid-containing foam cells. Metastatic growths from these tumors wrongly, but not uncommonly, impress the clinician as primary lesions. If such a metastasis occurs in an organ in which other xanthomatous changes are within the scope of the possible, the correct interpretation of a biopsy specimen becomes of utmost importance.

*Xanthoma Cells Occurring in the Vicinity of Inflammatory and Neoplastic Processes.*—Not infrequently typical lipid-containing foam cells are found in the walls of salpingitic abscesses (Pick), in the granulomas of leprosy and actinomycosis, in scars, in keloids, etc. Their formation is usually explained as a storage of lipoids in certain groups of connective tissue and blood cells (histiocytes) after cell deterioration has led to a local accumulation of these substances.

Dubs saw xanthoma cells developing from connective tissue cells in two cases of adenocarcinoma of the uterine body. There was neither tissue necrosis nor hypercholesteremia to explain the passive infiltrative lipid accumulation in the cells.

*Xanthoma Cells in the Mammary Gland.*—Xanthoma cells have been found in this organ with comparative frequency and in connection with various pathologic changes. Lobeck, in one hundred and sixteen amputated breasts, found areas of xanthomatosis nineteen times. The underlying pathologic changes included acute mastitis, benign tumors, malignant tumors and cystic disease. Careful microscopic and histochemical examination showed that foam cells may be formed either by connective tissue cells and adventitial cells of the blood vessels or by glandular epithelium and carcinoma cells. According to Lobeck, xanthomatous changes in the stroma are always indicative of a coexisting hypercholesteremia, while epithelial or cancerous formation of foam cells may occur without such metabolic abnormality. Hagensen classified the various findings of xanthoma cells in diseased breasts in three groups: (1) Primary xanthoma. Here he reported two observations of his own. The tumors were radiosensitive, amputation of the breast being carried out after marked clinical improvement. A third case was that of Cheatle, which Hagensen mentioned without giving details. A fourth case was that of a xanthomatous nodule at the site of a previous amputation of the breast for carcinoma. At the time of the biopsy, an enlargement of the lymph glands in the supraclavicular region was present, and radiologic observations in the skeleton made the diagnosis of generalized metastases rather certain. (2) Secondary xanthomatous degeneration in fibro-adenomas and carcinomas. (3) Xanthoma cells in traumatic fat necrosis of the breast.

Miller described a large and apparently benign tumor with numerous xanthoma cells in the connective tissue which he classified as cystosarcoma phylloides.

*Xanthoma of the Retroperitoneal and Mediastinal Spaces.*—Dietrich described a huge giant cell sarcoma originating in the vicinity of the suprarenal gland and infiltrating this organ and the adjacent kidney. Large numbers of lipid droplets were contained in spindle cells and in characteristic xanthoma cells. Malignancy was suggested by seemingly metastatic tumors of identical histologic structure in the orbits producing exophthalmos, at the base of the skull, in the peritoneum and in the myocardium. There is no reference to the blood cholesterol. Chiari properly suggested that this case might have been one of general xanthomatosis belonging in the category of the Hand-Schüller-Christian syndrome. A similar case reported by Nöthen was that of a tumor of the same localization and histologic appearance. The smaller tumors in the lungs, peritoneum and lymph nodes had a distribution somewhat more in line with a metastatic spread than those in Dietrich's case. Wessen removed an extraretropleural tumor growing with a broad pedicle from an intercostal space, which had a histologic structure of

the type seen in xanthomatous giant cell sarcoma. Heuer removed a xanthomatous tumor, well encapsulated and somewhat larger than a golf ball, from the posterior mediastinum. The point of origin from the thoracic wall could not be determined.

We pause here to summarize this complex, if not confusing, mass of pathologic data as follows:

1. In many cases, solitary xanthoma appears to be a special form of giant cell tumor.

2. Xanthoma cells may be found in neoplasms of epithelial and mesodermal genesis and in inflammatory cell infiltrations and proliferations.

3. The various clinical forms of multiple and generalized xanthoma are interrelated. The histologic pictures show little if any variation, and the occurrence of intermediary cases proves that the factor of localization in certain organs and systems cannot serve as a criterion for a scientific classification.

4. The survey of the pathologic anatomy does not lend support to the contention that multiple or generalized xanthoma can be etiologically explained as a phenomenon of reticulo-endothelial storage in the sense of a phagocytic accumulation of unphysiologic substances withdrawn from the blood stream. Two findings are not compatible with this theory: (*a*) the xanthomatosis of the tendons and tendon sheaths; (*b*) the extreme variability of the localization of xanthomatous changes. The phagocytosis of the reticulo-endothelial system is a physiologic function of normal cells. As shown by the experiments with vital staining, these cells respond to the introduction of foreign substances into the circulation in a typical and predictable way. If xanthomatous cell proliferations developed in a similar way, the great variation in the localization of xanthoma which accounts for so much of the complexity of the clinical aspects of this disease would remain unexplained.

## II. PATHOLOGIC PHYSIOLOGY

A humoral basis on which to rest the cellular changes found in xanthoma suggested itself to the first clinical observers of this disease. Addison and Gull, Fagge, and Pye-Smith remarked on the frequent association of xanthomatosis with jaundice. Gilbert and Lereboullet (quoted by Bross) committed themselves to the theory that cholemia produces xanthoma. To Quinquaud belongs the credit of having discovered the first essential facts of the blood chemistry in xanthomatosis. He found the fat content of the blood serum considerably increased in three patients and, according to Chambard, who collaborated with him in this investigation, recognized also an increase of the blood cholesterol. On the basis of these findings, Quinquaud explained the development

of xanthomas as inflammatory tissue reactions provoked by depositions of excess fatty substances in the circulation, the inflammatory tissue proliferation being modified by a reduced oxygen-combining power of the lipemic blood.

For a time disregarded by investigators like Török, Hallopeau and Hardaway, the significance of Quinquaud's studies was reestablished when Pinkus and Pick and Chauffard, Laroche and Grigaut pointed out the occurrence of excessive amounts of cholesterol compounds in foam cells and in the blood serum of patients with xanthoma. The etiologic relation between cholesteremia and xanthomatosis had now become the main issue of investigation. Unfortunately for a further advance in the search for a well established etiology, the blood chemistry in individual cases does not uniformly fit into any axiomatic scheme of a hypercholesteremia-xanthomatosis relationship. It is only fair to eliminate the cases of solitary xanthoma as proof for or against such a relationship, because not a few investigators regard these solitary neoplasms as essential tumors undergoing localized xanthomatous degeneration. Indeed, in the majority of instances of solitary xanthoma the cholesterol content of the blood seems to have been found within physiologic limits, although Weil reported high blood cholesterol also for these cases.

The difficulty in the way of establishing the hypercholesteremia theory arises from the inconsistency of the blood cholesterol findings in cases of generalized xanthoma. On the affirmative side may be listed the xanthomatoses complicating diabetes, icterus and, rarely, lipoid nephrosis. In these cases, without exception, high figures for blood cholesterol have been regularly reported. Furthermore, as a therapeutic test of this theory, involution of the xanthomas can unvaryingly be observed if successful antidiabetic treatment abolishes the concomitant lipemia and hypercholesteremia (Engman and Weiss; Major). Gaal could parallel these therapeutic results in a patient with multiple subcutaneous, tendinous and para-articular xanthomas and an "essential" hypercholesteremia by eliminating lipoids from the diet for a long time.

In the so-called essential xanthomatosis of the skin (xanthoma multiplex), a condition clinically quite similar to the icteric and diabetic xanthomatosis, reports of high blood cholesterol (for instance, by Mook and Weiss and Schmidt) are offset by an increasing number of reports of normal and subnormal levels of cholesterol (Rosenbloom; Rosenthal and Braunisch; Arzt; Siemens; Herrmann and Nathan; Greenbaum; Weidman; Baar; Wile, Eckstein and Curtis). In the Hand-Schüller-Christian syndrome an absence of increase in cholesterol has been observed in some cases (Rowland; Chester), although high values are

usually found during the more active stages of the disease. In Gaucher's disease no known changes occur in the chemistry of the blood. In Niemann-Pick's disease the blood cholesterol is often found increased, although the lipoid infiltration of the cells supposedly consists of phosphatid lipoids and not of cholesterol.

The frequency with which excessive concentrations of cholesterol in the blood are associated with xanthomatous deposits might serve as a crucial test of the hypercholesteremia theory. Such a comparison reveals a marked discrepancy between the frequency of hypercholesteremia and the development of xanthoma. Despite the fact that blood cholesterol reaches probably its highest concentrations in cases of obstructive jaundice (Wells; Leupold) xanthoma develops only in very few patients so afflicted. Diabetic patients with lipemia and hypercholesteremia, after all, do not show a high incidence of xanthoma. Excessive increases in cholesterol appear physiologically during the later months of pregnancy. Chauffard, Laroche and Grigaut found hypercholesteremia in 58 per cent of cases during the first seven months and in 94 per cent during the eighth and ninth month. This increase amounted in the average to 68 per cent, and occasionally surpassed 100 per cent: yet xanthoma is not common during pregnancy.

Westphal, who attributed to cholesterol an important rôle in the pathogenesis of essential arterial hypertension, stated that he found hypercholesteremia in more than seventy of one hundred patients, without making any reference to the existence of xanthoma. According to Joel, persons with a persistent and constitutional hypercholesteremia may remain free from symptoms and demonstrable pathologic manifestations for an indefinite length of time.

As a result of all this, many authors have either denied the etiologic importance of hypercholesteremia or qualified the theory with subtle physicochemical details. While it might be considered rather futile to dwell on the divergence of mere opinions, a brief résumé of these speculations may help to outline the field of further investigation.

Chvostek formulated his opinion as follows: "The essential factor for the infiltration of cells with lipoids is not a cholesteremia but a 'deconstitution' of the cells. A cholesteremia, if present, will only facilitate this process." The "deconstitution" of the cells he believed to be produced by sympathetic or hormonal stimuli. Rosenthal and Braunisch voiced the similar opinion that unknown changes in the chemism of the cells create an affinity for cholesterol, which is deposited intracellularly as ester compounds. Abrikossoff and Herzenberg, subscribing to the same theory, explained the frequent occurrence of hypercholesteremia in cases of xanthomatosis by a concomitant damage of the liver, which fails to eliminate cholesterol at the proper rate. Wile, Eckstein and

Curtis, on the basis of painstaking chemical analysis of blood and tissues, denied that cholesterol either in the blood or in the xanthomatous deposits plays the sole etiologic rôle and contended that a disturbance of the *cellular* fat metabolism more complicated and of unknown nature is at play.

While these interpretations emphasize deviations from the normal functions of cells as primary factors in the pathogenesis of xanthoma, other opinions still maintain that the morphologic changes are representative of the phenomena of storage, the foam and other cell proliferations being only automatic sequels of the unphysiologic concentration of cholesterol in the blood stream.

It can hardly be contended that the negative findings in regard to cholesterol rest on a failure to recognize that the concentration of cholesterol in the blood may vary during the course of the disease. Rosenbloom and Rosenthal and Braunisch made analyses of the blood during the acutely progressive stage in cases of cutaneous xanthomatosis, i. e., at a time when additional tumors were erupting, and they found normal and subnormal values for cholesterol even at this time, when one might reasonably expect changes in the blood to be most marked.

In order to account for the negative results of analysis of the blood in so many cases, and for the irregularity with which xanthoma develops in the presence of hypercholesteremia, a number of auxiliary hypotheses have been introduced. Herrmann and Nathan, following a suggestion of Handowsky, attempted to differentiate between serum cholesterol which can be directly extracted with ether and cholesterol that will go into solution only after protective serum colloids have been destroyed with potassium hydroxide. Assuming that an increase of the unprotected fraction without an increase of the total amount of cholesterol might explain the phagocytic storage in foam cells, they examined the serum of a patient with cutaneous xanthomatosis and normal total cholesterol in the blood. The results of this examination, however, did not lend support to the idea regarding extractable and nonextractable cholesterol.

Schaaf and Werner also expressed the belief that the merely quantitative increase of total lipoids is of minor importance in comparison with the altered quantitative relation of the various lipoid fractions to one another. Therefore they examined not only for cholesterol, but also for cholesterol esters, phosphatids, lecithin and neural fats. They found an increase of total cholesterol in only one of eight patients, but abnormal proportionality between the different lipoid and fat constituents of the serum in all of them. This they believe furnishes an explanation of the development of xanthoma in all those cases in which the quantitative determination of cholesterol and cholesterol esters alone gives no evi-

dence of a disturbed lipid metabolism. The changed quantitative relations between the different fractions of the serum lipid supposedly lead to diminished emulsification, because they influence one another in their physicochemical behavior. The changed magnitude of dispersion units provokes phagocytic reactions of certain tissue cells.

Weidman suggested that the present chemical methods of analyzing blood and tissue probably do not succeed in discovering the chemical basis of xanthomatosis. The lipid substances that one finds at present in blood and tissue may only accompany the chemical agent. Weidman referred to the evolution of the knowledge of ergosterol and vitamin D, which could be isolated only in minute quantities from cholesterol and other lipoids with which they always appear together. However hypothetical this comparison may be, it is true that both the histochemical findings in xanthoma and the blood chemistry in hypercholesteremia are more complex than the emphasis on cholesterol suggests. In the tissues, lipochrome and hemosiderin pigments can be found in most of the instances; sometimes the foam cells store lipid substances other than cholesterol esters, as in Niemann-Pick's disease and Gaucher's disease. Neutral fat has been found by Fahr and Chvostek. The blood changes, on the other hand, involve the other lipid fractions, too, because in every case of hypercholesteremia an increase of the neutral fat in the blood serum and often of other lipoids can be found. From all these facts Weidman's suggestion gains support that from the complex mixture of so-called lipid substances participating in the pathologic changes only few fractions are known and many more may still remain to be discovered.

While the research into the nature of xanthomatosis still falls short of furnishing enough accurate data on which to evaluate properly the rôle of hypercholesteremia, Schönheimer has recently tried to determine how the accumulation of cholesterol in the blood plasma is brought about. He based his examinations on the following considerations: Cholesterol in the organism is either absorbed from animal food or synthesized from metabolic products. After being excreted through bile and the intestinal mucosa, it is reabsorbed, unless it has been transformed into dihydrocholesterol, which is nonabsorbable. Lipoids in vegetable food are nonabsorbable. Under both a mixed and a vegetable dietary regimen the organism excretes cholesterol and dihydrocholesterol into the intestinal tract, cholesterol being reabsorbed to a great extent while dihydrocholesterol is finally excreted in greater quantities. Schönheimer's patient suffered from a lipemia of 2,100 mg. and a hypercholesteremia of 825 mg. per hundred cubic centimeters. Multiple xanthomatous tumors were present in tendons and tendon sheaths, and the scar of an old laparotomy had undergone xanthomatous changes. When the patient was put on a vegetable diet with exclusion of absorb-



able lipoids, the blood cholesterol dropped to 300 mg. per hundred cubic centimeters, but there was no influence on the xanthomatosis. The feces showed a lack of cholesterol and cholesterol derivatives on both a mixed and a vegetarian diet, while dihydrocholesterol could be found up to the unusually high concentration of 10 per cent of the total lipoids in the blood plasma. Schönheimer came to the conclusion that his patient was able to absorb cholesterol from the food, but unable to excrete it. The accumulation of dihydrocholesterol in the blood was an additional proof of deficient excretory function.

Recourse has been had repeatedly to animal experimentation in order to reproduce xanthoma formation by overloading the circulation with lipoids. The basis for this experimental work was furnished by the experience accumulated during the investigation of artificially produced hypertension and atherosclerosis. In the pursuit of those studies it was discovered that herbivorous animals, the rabbit in particular, acquire this condition after feeding on various animal food substances in which cholesterol could be found as the pathogenic agent; feeding with pure cholesterol (Versé) yielded identical results, and hypercholesteremia invariably preceded the hypertension. The anatomic changes found in the hypercholesteremic rabbits were not confined to the arteries, but involved many parenchymatous organs and aggregates of reticulo-endothelial cells, which sometimes assumed the shape of typical foam cells infiltrated with cholesterol esters. The general well-being of the animals was greatly impaired, so that Schönheimer spoke of an "experimental cholesterol disease of the rabbit."

Anitschkow reproduced xanthoma cells in the experiment by creating sterile abscesses in the subcutaneous tissue of hypercholesteremic rabbits. In the granulation tissue limiting these abscesses typical foam cells with doubly refractive lipoids were found.

Weidman was unable to confirm Anitschkow's results. He, furthermore, experimented on hypercholesteremic and on xanthomatous patients by inflicting operative injuries and applying chemical irritation. None of his patients showed xanthomatous scars.

Wustmann injected silicious earth close to the bones of hypercholesteremic rabbits. The granulomas contained cholesterol deposits, but not cells comparable with xanthoma cells.

Hoessli experimented with hypercholesteremic rabbits. The tendo achillis was concussed for eight minutes daily for from four to six weeks. The tendon showed infiltration with cholesterol but no proliferation of foam cells. By no means did the histologic structure resemble the xanthomatous tumors of the tendons of a patient of his who had hypercholesteremia. Lebedew experimented by inserting sterile foreign bodies like cork and sutures saturated with 10 per cent potassium hydroxide into the subcutis of hypercholesteremic rabbits. The first results were negative, but after prolonging the irritation and examining the tissues at a later time, when the inflammation had become more chronic, he could observe typical foam cells containing lipid droplets. Experiments on normal rabbits were carried out by

making local deposits of cholesterol dissolved in sunflower oil. Here again histologic structures resembling xanthofibromas could be discovered from two and a half to three months after the injection of cholesterol.

Basten used an emulsified gallstone for local deposits in the ears and subcutis of a normal rabbit. He found large pale cells containing cholesterol esters as early as seven days after injection. These cells were derived from connective tissue cells and contained cholesterol ester.

As interesting as these experimental results may be, they are not uniform nor are they obtained under conditions that parallel human disorders sufficiently to make them applicable to the problem of xanthoma. In this connection it might be necessary again to emphasize certain differences in the lipid metabolism of the rabbit and the human being which have been brought out by the extensive experimental work with cholesterol feeding. Man and omnivorous animals react to an alimentary excess of fat with a transitory and visible lipemia; i. e., the blood plasma becomes milky because of the accumulation of fat droplets. On repetition of the heavy doses of fatty substances the organism gains corrective faculties and soon no visible or concealed lipemia of greater extent occurs. The opposite behavior is observed in herbivorous animals. Here a single fat feeding does not produce lipemia, but a prolonged feeding with fats in combination with cholesterol eventually produces a lipemic condition, which at first can be detected only by chemical determination, but later on is disclosed by a clouding of the blood plasma. Feeding cholesterol alone or feeding neutral fats alone is not conducive to a visible lipemia. But every increase of cholesterol in the blood is associated with an increase of neutral fats regardless of the source of the hypercholesteremia. Likewise, any lipemia caused by pathologic metabolism or by overfeeding with neutral fats is accompanied by a rise of blood cholesterol. These findings point to a complexity of the metabolism of lipid substances and create considerable doubt as to whether cholesterol has an independent metabolism. They suggest further that lipoids may circulate emulsified in varying degree, sometimes coarse enough to become visible in the blood plasma, sometimes in very fine droplets not interfering with its transparency. These facts have also been exploited for the theory of the etiology of xanthoma.

Experimental work has furthermore been carried out with regard to the histogenesis of foam cells. Biedermann and Höfer studied, in tissue culture, a specimen of bone marrow from a patient with Hand-Christian-Schüller's disease whose blood showed 238 mg. of cholesterol per hundred cubic centimeters. The appearance of the growing cells suggested their origin from the reticulo-endothelium. They developed into typical foam cells. This tissue culture was prepared with the hypercholesteremic serum of the patient. Fibroblasts from chickens grown in

the same medium failed to develop into foam cells. This indicates that the chemical medium is not so important a factor as are the essential metabolic characteristics of the cells themselves.

Summarizing the foregoing histochemical discussion, we may conclude that:

1. Unphysiologic concentrations of cholesterol or cholesterol esters in the blood occurring either primarily or secondarily to other known humoral disorders are sometimes associated (but not necessarily so) with xanthomatous foam cell tumors or granulomas.

2. Xanthomatous tissue changes may occur without increase of cholesterol in the blood.

3. Several attempts have been made by various authors to defend the theory of the etiologic relation between blood cholesterol and xanthoma in spite of the fact that hypercholesteremia is not apparent in a considerable number of typical cases. Other investigators deny such an etiologic relationship.

4. In diabetic xanthomatosis an involution of the xanthomatous lesions can be regularly brought about by antidiabetic treatment that occasions a lowering of the lipcholesteremia. In other forms of xanthomatosis, however, although the accompanying lipcholesteremia may sometimes be favorably influenced by a prolonged dietary regimen, an improvement of the pathologic tissue changes may not necessarily follow.

5. The etiology of various forms or types of xanthomatosis is still to be determined.

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# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

INACTIVATION OF INSULIN BY NORMAL AND DIABETIC BLOOD. PETER T. BLACK, Brit. J. Exper. Path. **14**:318, 1933.

Insulin incubated with serum or with laked cells or with cytolysed leukocytes loses some part of its power to lower the blood sugar and produces convulsions in fasting rabbits when the mixture is injected subcutaneously. Insulin incubated with laked cells loses some part of its power to lower the blood sugar when injected intravenously into rabbits. This is probably due to destruction of the insulin by an enzyme other than trypsin. The inactivating substance does not readily pass from the cells to the plasma. Insulin incubated with heparinized whole blood retains its power to lower the blood sugar when injected intravenously into rabbits. This is also true of insulin incubated with intact red cells or leukocytes. Blood from diabetic patients, even from those displaying resistance to insulin, had no more than normal power to destroy insulin in any of these experiments. This investigation, therefore, does not support the view that the symptoms of diabetes are due to destruction of insulin in the circulation.

AUTHOR'S SUMMARY.

BIOLOGIC TEST FOR HODGKIN'S DISEASE. C. E. VAN ROOYEN, Brit. M. J. **1**:644, 1933.

Gordon found that the intracerebral inoculation of rabbits and guinea-pigs with suspensions of lymph glands from persons with Hodgkin's disease was followed in a few days by spastic paralysis of the hindlimbs, rigidity, ataxia and muscular weakness. Of five cases of Hodgkin's disease in which this test was made, there were three in which a positive reaction was observed; the reaction in one was doubtful and in one, negative. The reaction cannot be elicited by the use of normal, lymphosarcomatous, leukemic or tuberculous lymphatic tissue. Neither can it be brought about by the intracerebral inoculation of dead bacteria, streptococcus toxin, sterile milk, peptone water, sodium nucleate or finely powdered glass.

JACOB KLEIN.

SOME EFFECTS OF ARTIFICIAL PNEUMOTHORAX ON THE CIRCULATION. R. HILTON, J. Path. & Bact. **37**:1, 1933.

It is suggested that the cause of the first increase in minute volume observed after the induction of pneumothorax is due to the increased inspiratory efforts and probably to anoxemia. The subsequent fall in minute volume may be explained by a diminished mechanical efficiency of the breathing when the pleural cavity is full of air, with a consequent interference with the return of venous blood to the heart.

AUTHOR'S SUMMARY.

EFFECT OF EXPERIMENTAL PORTAL CONGESTION ON THE ABSORPTION AND EXCRETION OF WATER. J. McMICHAEL and F. H. SMIRK, J. Path. & Bact. **37**:81, 1933.

Rats in which the portal vein has been obstructed show, on the day after operation, a diminished rate of absorption of water from the alimentary tract and a delayed diuresis. Venous anastomoses open up in the course of the week following operation, and the delayed absorption rate improves but does not return to normal. The diuresis curve may at this time have returned to normal despite the moderate delay in the absorption of water

AUTHORS' SUMMARY.

THE FUNCTIONS OF BRUNNER'S GLANDS AND THE PYLORIC END OF THE STOMACH. H. W. FLOREY and H. E. HARDING, *J. Path. & Bact.* **37**:431, 1933.

A description is given of a method of collecting a considerable amount of the secretion of Brunner's glands in the rabbit. This secretion is very alkaline— $p_H$  8, rapidly rising to  $p_H$  9 on exposure to air—and is at least equivalent to tenth-normal sodium bicarbonate, and in most cases more. The alkalinity is due to its content of bicarbonates. The fluid is of the consistency of the white of egg and clear or slightly opalescent. Brunner's glands of the rabbit, cat, guinea-pig and rat are activated by passing hydrochloric acid over them. Those of the rabbit respond to stimulation with oil of mustard. The relationship of this activity to the extrinsic innervation is discussed. The significance of this alkaline secretion for the protection of the gastric and duodenal mucosae is indicated, and a possible relationship between a failure of secretion and the production of peptic ulcer is suggested.

AUTHORS' SUMMARY.

INFLUENCE OF COLLOIDAL IRON AND ALTITUDE ON EXPERIMENTAL ANEMIA. WALTER HEIMANN, *Beitr. z. Klin. d. Tuberk.* **82**:418, 1933.

Anemia was induced in rabbits by the injection of acetylphenylhydrazine. The rate of spontaneous recovery was then compared with that following intravenous administration of active colloidal iron oxide. No significant acceleration of regeneration was observed. The experiment was repeated at an altitude of 3,000 meters and also in a low pressure chamber. In both, spontaneous recovery occurred at twice the rate of that in the lowlands. Again, colloidal iron oxide administered intravenously did not accelerate the process. In fact, in large doses it definitely hindered regeneration.

AARON EDWIN MARGULIS.

TISSUE CULTURE STUDIES ON BLOOD VESSELS AND SEROUS MEMBRANES. WERNER SCHOPPER, *Beitr. z. path. Anat. u. z. allg. Path.* **88**:451, 1932.

Tissue was obtained from guinea-pigs, and the cover glass method of Maximow was followed. As a medium, plasma of guinea-pigs with heparin and embryonal extract was used. Normal omentum, omentum after the injection of infusorial earth, vena cava and aorta were studied. Small blood vessels were obtained from the leptomeninges. The tissue cultures were observed during from ten to twelve subcultures.

The growth of the serous cells lining the surface of the omentum in the early cultures is epithelial-like. They form membranes consisting of large, flat, surface cells. In later stages, they more and more resemble fibroblasts. In tissue defects, the serous cells do not regenerate from the fibroblasts of the omentum. The undifferentiated mesenchymal cells which are distributed diffusely in the omentum and the adventitial cells surrounding capillaries form macrophages which are able to engulf neutral red. Under certain conditions, the macrophages may develop into fibroblasts.

Small lymphocytes emigrating from the sheaths of the blood vessels degenerate after a few days. Buds of endothelial cells grow out of capillaries into the tissue and into the surrounding culture medium. These buds are mostly solid and consist of slender endothelial cells. In later cultures single endothelial cells are found, resembling fibroblasts. The muscle tissue of the larger blood vessels in the omentum does not proliferate, but may be seen well preserved for thirty-five days.

If infusorial earth is added to the tissue culture in too large amounts, cell proliferation will be stopped. Also, small doses will lessen the epithelial-like growth of the serous cells. The emigration of macrophages is, however, increased. Foreign body giant cells form by fusion of macrophages. Typical foreign body tubercles surround deposits of infusorial earth. The omentum obtained from animals after intraperitoneal injection of infusorial earth shows a much more active



cell proliferation than in normal tissue. There are many foreign body giant cells, wandering cells, lymphocytes and serous cells present. The serous cells grow either as an epithelial-like membrane or as fibroblasts.

In cultures of aorta and vena cava, the adventitial cells grow first in a circular arrangement; later they grow into the medium like typical fibroblasts and develop fine fibrils. The latter originate only from cells. The endothelial cells grow into the lumen and may cause obliteration of the lumen by producing fibrils.

The cerebral arteries were dissected carefully from the surrounding tissue prior to culture. The endothelial cells are very active, producing solid and tubular buds which may form a network. Small artificial defects in the wall of the vessel are filled out by proliferating endothelial cells. If a blood vessel is cut in two, the stumps will unite, if these are lying close together. If the stumps are far apart, union is made impossible because adventitial cells will grow into the defect before endothelial cells show proliferation.

C. ALEXANDER HELLWIG.

THE PROBLEM OF JAUNDICE CAUSED BY TOLYLENEDIAMINE. H. H. GREENE and W. SCHAAL, Beitr. z. path. Anat. u. z. allg. Path. **89**:61, 1932.

In dogs, injection of tolylenediamine caused hemolysis and also degeneration of the intrahepatic bile ducts. The degree of hemolysis is less after removal of the spleen. The jaundice occurring after the injection of tolylenediamine apparently has three explanations: 1. It may be due to the degeneration of the epithelial cells of the intrahepatic bile ducts. The bile excreted by the liver cells thus passes into the hepatic lymph spaces and from there directly or by way of the thoracic duct into the blood stream. 2. Hemolysis may play a causative rôle in the jaundice, as evidenced by the marked swelling of the Kupffer cells and their phagocytosis of the debris of erythrocytes. 3. The formation of bile thrombi in the bile capillaries of the liver may cause true obstructive jaundice.

C. ALEXANDER HELLWIG.

INFLUENCE OF SALT SOLUTIONS ON THE RENAL TISSUE. H. NIEWIESCH, Beitr. z. path. Anat. u. z. allg. Path. **89**:76, 1932.

Different salt solutions, in various concentrations, were injected into the left renal artery of rabbits and the histologic changes in the left kidney were compared with the normal structure of the other kidney. Calcium chloride, sodium chloride and sodium phosphate (monobasic) produced coagulation necrosis of the epithelial lining of the convoluted tubules. By using higher concentrations of these substances, degeneration of the straight tubules was noticed, whereas the glomeruli were most resistant. A marked leukocytic infiltration was observed in the interstitial tissue surrounding the degenerated tubules. These inflammatory changes are, in the opinion of the author, directly due to irritation by the chemical substances and precede the necrosis of the tubules.

C. ALEXANDER HELLWIG.

EXPERIMENTAL STREPTOCOCCIC ARTHRITIS IN RABBITS. G. WEHRSIG and A. J. WEIL, Beitr. z. path. Anat. u. z. allg. Path. **89**:311, 1932.

Rabbits were given intravenous or intraperitoneal injections of various strains of streptococci. Single and repeated injections, with and without previous sensitization by vaccine, were made, and the resulting lesions in the joints were studied microscopically. The arthritis produced in rabbits by the injection of streptococci did not show the microscopic picture of infectious rheumatism, but resembled septic arthritis as found in man after general infection with pyogenic organisms. The lesions of the joints originated either from the capsule or from the bone marrow beneath the cartilaginous surface of the joint.

C. ALEXANDER HELLWIG.

GLYCOGENOSIS. P. KIMMELSTIEL, *Beitr. z. path. Anat. u. z. allg. Path.* **91**:1, 1933.

The condition which the author portrays was first described under the name glycogenic hepatonephromegaly by von Gierke in 1929 (*Beitr. z. path. Anat. u. z. allg. Path.* **82**:497, 1929; abstr., *ARCH. PATH.* **10**:125, 1930); two additional cases were reported by Putschar (*Beitr. z. path. Anat. u. z. allg. Path.* **90**:222, 1932; abstr., *ARCH. PATH.* **17**:4, 1934). The author's case is the fourth recorded example of this condition, for which he prefers the name "glycogenosis." A child died of acute pharyngitis and bronchopneumonia; the age at death is not stated in the brief clinical summary. Enlargement of the abdomen was noticed by the mother when the child was between 3 and 4 weeks old. At necropsy, the liver extended below the level of the umbilicus and weighed 1,600 Gm. Each kidney weighed 45 Gm., and, as was true in Putschar's cases, was much less enlarged than in von Gierke's case. The liver, cardiac muscle, voluntary muscle and brain contained large quantities of glycogen; the kidneys contained a smaller amount, situated chiefly in the epithelium of the straight tubules. The glycogen did not disappear as rapidly from the liver after death as is usually the case. Although the glycogen of the liver yielded as large a proportion of dextrose on acid hydrolysis as did pure glycogen or the glycogen of normal dog's liver, it yielded a definitely smaller proportion when subjected to diastatic fermentation. The pancreatic islets were histologically normal with the exception of a few that were somewhat enlarged. The two previous observers sought the explanation of the condition in decreased glycogenolytic activity of the liver. In view of the unequal distribution of the glycogen, especially in a single organ like the brain, Kimmelstiel does not accept this suggestion. He postulates a disturbance of carbohydrate metabolism that leads to the formation of a heterotypical form of dextrose, which is polymerized into a form of glycogen that is not readily split by the normal diastatic enzyme.

O. T. SCHULTZ.

### Pathologic Anatomy

SPLENOMEGALY AND SPLANCHNOMEGALY. M. MÜHLMANN, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:180, 1932.

The author reports a case in which marked enlargement of the spleen was associated with increase in the size of the liver, kidneys, testes and lungs, without evidences of acromegaly. The enlargement of the organs was due to hypertrophy of the parenchymatous elements, to an increase in the amount of collagenous, elastic and reticular fibrillated tissues, and to an increase in the size of the cells of the reticulo-endothelial system. The lymphatic elements were atrophic. The globus pallidus of the left lenticulate nucleus was about one-third larger than that of the right. Microscopically, it revealed an increased number of glia cells, as compared with the right globus, and a paucity of ganglion cells, which were numerous and large on the right side. Mühlmann then presents epitomes of four additional cases of what he holds to be the same condition, although insufficient attention was paid to the brain. Finally, he gives brief summaries of sixteen cases collected during his earlier work in Baku, which cases he had at that time looked on as Banti's disease. Splanchnomegaly, of which splenomegaly is only a part, is a constitutional systemic disease in which the hypertrophy of the organ is due to abnormal functioning of the central vegetative nervous system. This conception is supported by Mühlmann's previously propounded theory that the hyperplasia of tumor formation is the result of the activity of the central vegetative nervous system, for which theory, it may be parenthetically remarked, there does not seem to be any strong basis of support.

O. T. SCHULTZ.

CONGENITAL GLYCOGENIC HYPERTROPHY OF THE HEART. W. PUTSCHAR, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:222, 1932.

A child died at the age of 4 months of bilateral bronchopneumonia. At the age of 12 days, generalized edema was noted, but no abnormality of the heart was detected. At 13 weeks of age, it was noted that the liver was enlarged, and on

admission to the hospital marked enlargement of the heart was detected. At necropsy, the heart was found greatly enlarged, weighing 110 Gm. The wall of the left ventricle varied from 7 to 21 mm. in thickness, that of the right ventricle from 6 to 12 mm. The liver was moderately enlarged and weighed 240 Gm. Each kidney weighed 25 Gm. The increase in the size of the heart was due to marked infiltration of the muscle fibers by glycogen. The liver cells and the epithelium of the convoluted tubules of the kidney were vacuolated by glycogen. The case is held to belong to a group of which von Gierke described two examples in 1929 as congenital glycogenic hepatonephromegaly. Von Gierke considered the condition one of abnormal glycogen metabolism, which he likened to the diseases characterized by lipoid infiltration, namely, Gaucher's disease, Niemann-Pick's disease and Hand's (Schüller-Christian's) disease. In Putschar's report there is no note of histologic examination of the pancreas. In a brief note he mentions the case of a 3 year old child in whom abnormal carbohydrate metabolism was detected during life by chemical studies. Biopsy revealed that the enlargement of the liver detected clinically was due to glycogen infiltration.

O. T. SCHULTZ.

OSSIFICATION OF THE SACRAL INTERVERTEBRAL DISKS. A. S. JACOBSON and H. DRIVER, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:233, 1932.

The work here reported comes from Schmorl's institute, where the vertebral column is removed and subjected to study as a routine procedure. It is based on the study of 258 sacrums of persons of the second to ninth decades, inclusive. The anatomists cited state that ossification of the sacral intervertebral disks begins during the eighteenth year of life and usually leads to bony union of the vertebral bodies by the twenty-fifth year. The authors found that ossification was already evident during the second decade of life, but that even so late as the ninth decade the process was not complete. Ossification became progressively more marked in a caudal direction. Complete ossification of the first, second and third disks was noted in no instance during the second decade and occurred in 10, 46 and 53 per cent, respectively, in the ninth decade. Only the three upper disks are included in the tabulations, because the fourth and fifth sacral vertebrae, were not removed in every case. Partial and complete ossification of the first, second and third disks was observed in 4, 15 and 25 per cent, respectively, during the second decade, in 65, 89 and 100 per cent during the eighth decade and in 75, 100 and 100 per cent during the ninth decade. Ossification is due not to the ingrowth of blood vessels into the disks, but to the invasion of the disks by the marrow of the vertebral bodies. The disk is then transformed partly or completely into spongy bone. The process is looked on as phylogenetic.

O. T. SCHULTZ.

THE NEUROMUSCULAR APPARATUS OF THE APPENDIX IN CHRONIC APPENDICITIS. L. SCHACK, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:392, 1932.

Schack's description of changes in the neuromuscular apparatus of the appendix is preceded by a description of this apparatus in the normal organ. The technical methods of Masson were used, and this author's conception of the normal neuromuscular apparatus is confirmed. The apparatus consists of four parts: the external and internal muscular coats with the plexus of Auerbach; the musculonervous complex of the submucosa, which includes Meissner's plexus; the muscularis mucosae, which has its own nerve plexus, and the mucosa with the periglandular plexus of Masson. Seventy-six of 150 chronic appendixes taken at random revealed changes in the neuromuscular apparatus similar to those described by Masson in what the latter considered a characteristic type of appendical involvement which he termed neurogenous appendicitis. The changes noted were hypertrophy or localized hyperplasia of the neuromuscular apparatus. Such changes were always associated with other changes that were the result of previous active inflammation. Schack therefore contends that Masson's neurogenous appendicitis is not a specific type of involvement, but is a result of inflammation.

Hypertrophy of the neuromuscular apparatus is a work hypertrophy, brought about by increased resistance to movement of lymph and secretion resulting from structural alterations caused by inflammation. The localized hyperplasias are minute neuromas that are the result of the inflammatory stimulus. They correspond to the small neuromas frequently seen in the chronically inflamed submucosa of the gallbladder and stomach. Schack detected axis-cylinders in such neuromas and observed degenerative changes of the neurofibrils at the center of the neuromas. There was nothing characteristic in the clinical symptomatology that might be correlated with the changes in the neuromuscular apparatus.

O. T. SCHULTZ.

THE CHROMAFFIN CELLS OF THE APPENDIX. L. SCHACK, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:441, 1932.

In a profusely illustrated article, Schack discusses the embryogenesis, the emigration, the topography, the morphology, the relationship to muscular and nerve tissues, the quantitative distribution in various types of appendicitis, the special pathologic process and the oncology of the chromaffin cells of the appendix. In the fetal appendix the cells are seen first in the epithelium of the crypts and are of epithelial origin. They emigrate early into the neuro-epithelial syncytium, the presence of which was first described by Masson and is confirmed by Schack, at the bottom of the crypts. From here the cells wander more deeply into the mucosa, retaining a relationship to the neuromuscular apparatus. In the normal appendix they occur in small numbers in the muscularis mucosae, and a few are seen in the submucosa. Single cells have the morphology of ganglion or glia cells. They are impregnated by silver and stained by chrome salts and by acid fuchsin. The cells belong to a group that includes the chromaffin cells of the suprarenal medulla and the melanophores of the skin. The chromaffin cells of the appendix are believed to have an endocrine function which acts on that portion of the neuromuscular apparatus not concerned in the emptying of the appendix. The neurotropic action of the cells manifests itself in growth of neurofibrils. The cells are increased in number in the pathologic appendix, but only when the changes are such as to warrant the conclusion that the appendix has been previously inflamed. These changes include the neuromuscular hypertrophy to which Schack has called attention in a preceding article (*Beitr. z. path. Anat. u. z. allg. Path.* **90**:392, 1932). In the localized neuromuscular hyperplasia of the obliterated appendix, the chromaffin cells are increased in number in the neuromatous areas. In a carcinoid of the appendix, which is considered a tumor-like maldevelopment comparable to nevus of the skin, the neurotropic action of the chromaffin cells was evident by their growth in and along Auerbach's plexus and by increased neurofibril formation in the plexus.

O. T. SCHULTZ.

RELATIVE FREQUENCY OF CALCIFICATION OF THE MITRAL RING. G. MARTENS, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:497, 1932.

The relative frequency of deposition of calcium in the mitral ring was studied in 1,000 hearts, equally distributed between the two sexes. Calcification was detected in 87 (8.7 per cent) of the hearts. It occurred three times as frequently in women as in men, and was noted at an earlier age in women, 54 years for women as compared with 59 years for men. The condition reached its highest frequency in the age period of from 71 to 74 years. Calcification of the mitral ring was associated with hyperostosis of the frontal bone and calcification of the intervertebral disks. The three processes are looked on as regressive changes, which are probably the result of alterations in calcium metabolism that occur with advancing years.

O. T. SCHULTZ.

THROMBOSIS OF THE HEPATIC VEIN. L. BERK, Beitr. z. path. Anat. u. z. allg. Path. **90**:509, 1932.

The two cases briefly reported, both in women aged 41, were identical clinically and pathologically. In each there were ascites, edema of the lower extremities and a visible collateral venous circulation of the abdomen. At necropsy, there was found organizing mural thrombosis of a portion of the inferior cava 2 cm. long at the region of the mouth of the hepatic vein. The cava was occluded by fresh thrombus, which in one case extended into the iliac and femoral veins. The hepatic vein in each instance was occluded by fresh thrombus. The portal vein was not thrombosed. Syphilis was excluded in each case. These cases differ from the primary endophlebitic thrombosis of the hepatic vein described by Chiari, in that the thrombosis of the hepatic vein was secondary to an older, localized thrombotic process in the cava. As the cause of the latter process, mild trauma is postulated, such as might arise from attacks of coughing.

O. T. SCHULTZ.

EARLY CHANGES AND EVIDENCES OF LATE RESORPTION IN THE LESIONS OF GOUT. G. POMMER, Beitr. z. path. Anat. u. z. allg. Path. **90**:513, 1933.

Each of the first eight paragraphs, which together make up two of the seven pages of Pommer's article, consists of but a single sentence, a fact that should make for easy reading were it not for the further facts that each of these paragraphic sentences consists of from twenty-five to sixty-seven words and that each sentence contains from three to seven compound German words of from fifteen to twenty-five letters each. So much for statistics, which drove the abstractor to a rereading of "Sartor Resartus" and to renewed acquaintance with Herr Teufelsdröck. Pommer describes the early reactions and some late resorptive changes of the gouty lesion, which he believes did not receive adequate description in his "Gelenkgichtuntersuchungenmonographie." In the early lesions it is necessary to distinguish between those that arise between the fiber bundles of the tendons and those that arise within the bundles. The interfascicular deposition of urates leads to a productive inflammatory reaction about the deposit. In the intrafascicular deposition of urates, such inflammatory reaction is absent until the lesion reaches such a size that it encroaches on the interfascicular tissue. In older lesions that have undergone partial fibrosis or calcification, or even ossification, two forms of resorption are evident. Lacunar resorption, associated with the formation of foreign body giant cells, occurs at the surface of the deposit. In other instances, the mass becomes hollowed out or canalized by central resorption.

O. T. SCHULTZ.

COMPRESSION OF THE CORD BY A VERTEBRAL HEMANGIOMA. E. SCHERER, Beitr. z. path. Anat. u. z. allg. Path. **90**:521, 1933.

Small, sometimes multiple, hemangiomas of the vertebral bodies were noted in 409 (10.7 per cent) of 3,829 vertebral columns examined in the Schmorl institute. These lesions produce no clinical effects. More widespread hemangiomatous involvement of a vertebral body leading to compression of the cord is relatively rare. The author summarizes twelve such cases from the literature and adds one of his own. The patient was a woman, aged 59, who began to be troubled with pain and weakness of the lower extremities two months before hospitalization for operation. On admission, there was complete sensory and motor paralysis below the tenth dorsal segment. The roentgenographic diagnosis was hemangioma of the seventh thoracic vertebra. Resection of the laminae and spinous processes of the sixth, seventh and eighth vertebrae, which had been preceded on the previous day by heat coagulation, resulted in slight improvement, but within eight weeks paralysis was again as complete as before the operation. Death occurred four months after operation. The portions of the seventh vertebra removed at operation and the rest of the vertebra removed at necropsy were the seat of a cavernous hemangioma that had led to atrophy of some of the spongy osseous trabeculae and

to the formation of osteoid tissue. The spinal canal was greatly narrowed. The dura was not involved. Transformation of capillaries into cavernous spaces was evident in the peripheral portions of the hemangioma. Formation of vessels by endothelial budding and transformation of the mesenchyme of the marrow into vessels were not seen.

O. T. SCHULTZ.

FIBROUS INDURATION OF THE HILAR LYMPH NODES OF THE LUNG. W. GIESE, *Beitr. z. path. Anat. u. z. allg. Path.* **90**:555, 1933.

A review of the literature relating to silicosis and indurative tuberculosis of the lung precedes the detailed description and discussion of Giese's study of induration of the bronchial lymph nodes. The findings that he reports are based in the main on the histologic study of human nodes and lungs, but in part also on experimentation on animals. Fibrous induration of the bronchial nodes may be due either to silicosis or to tuberculosis, the two processes being readily distinguished microscopically. The two processes may be combined. Inoculation into animals revealed that in every instance in which tuberculosis developed in the animal the tuberculous character of the process in the human nodes could be detected histologically. Silicosis may lead to nodular or diffuse induration of the nodes, the former being the result of the transport to the nodes of larger amounts of stone dust. The process begins as reticulo-endothelial hyperplasia in the subcapsular follicles, which are transformed into hyaline, densely fibrous nodules. Diffuse induration, which is due to fibrosis of the reticulum, may be associated with the nodular form or may occur alone; the latter results from small quantities of stone dust. The inorganic ash of incinerated nodes was from five to ten times greater in silicotic and tuberculous indurated nodes than in normal nodes of the same age period. The high ash content of tuberculous indurated nodes is due to the chronicity of the process and to increased phagocytosis of dust by the tuberculous granulation tissue. In both silicotic and tuberculous nodes, the dense fibrous tissue may undergo degeneration, softening and organization or calcification. The pleural lymph nodes undergo the same changes as the hilar nodes; in both, silicotic induration can be differentiated histologically from that due to tuberculosis. Perforation of an indurated node into a bronchus is usually due to tuberculosis, although the latter process may have been combined in the node with silicosis. The transportation of stone dust to the liver and spleen occurs by way of the blood stream and results from the passage of dust into the small vessels of the lung, or from perforation of a vessel by a silicotic hilar node. In the spleen silicosis may lead to induration of the follicles similar to that of the bronchial nodes. Tuberculous induration of the bronchial nodes was found by Giese to be more frequent than the silicotic form. Silicotic induration of the bronchial nodes depends on the physical and chemical properties of the inhaled stone dust and may occur unassociated with silicosis of the lung.

O. T. SCHULTZ.

CONGENITAL ANOMALY OF THE HEART WITH TRANSPOSITION OF THE AORTA. L. KETTLER, *Virchows Arch. f. path. Anat.* **287**:10, 1932.

The heart, which is described in detail, came from a child that died at the age of 15 months. The clinical diagnosis during life had been congenital cardiac anomaly, with a surmise of defective ventricular septum and open ductus arteriosus. The auricular and ventricular septums were defective. There were three ventricular cavities. The third ventricle, which gave rise to a pulmonary artery with only two valve segments, had been formed by division of the right ventricle by the persistent septum of the bulbus arteriosus. There was no right auriculoventricular orifice; the left had five valve segments. The aorta arose from the right ventricle; the coronary arteries were transposed. The left superior cava was persistent; the right had disappeared. The pulmonary veins entered the heart by a single trunk. There was a persistent coronary sinus with two valves.

O. T. SCHULTZ.

SYNCYTIAL STRUCTURE OF GLIA. W. G. LAZAREW, *Virchows Arch. f. path. Anat.* **287**:227, 1932.

After reviewing the differences of opinion that have been held on the question whether normal glia has a syncytial structure or is composed of individualized cells, the author presents the results of a study of pathologic material derived from cerebral cysticercus, progressive paralysis and glioma of the brain. He concludes that in these conditions, at least, the glia has a syncytial structure. The nuclei of the syncytium have a limited degree of mobility.

O. T. SCHULTZ.

CYSTS OF THE POSTERIOR ROOTS OF THE SPINAL NERVES. U. HINRICHS, *Virchows Arch. f. path. Anat.* **287**:242, 1932.

As a chance observation at necropsy the author describes a pea-sized cyst situated at the point of union of the ganglion with the posterior root of three different spinal nerves. The patient was a woman, aged 61. The origin of the cysts could not be determined.

O. T. SCHULTZ.

HISTOLOGY OF HUMAN APOCRINE SWEAT GLANDS. W. RICHTER, *Virchows Arch. f. path. Anat.* **287**:277, 1932.

This study is based on a series of 106 autopsies in which the apocrine glands of the skin were subjected to a careful histologic examination as to their content of iron, fat and cholesterol. All of the apocrine glands examined were found to contain iron. Evidence is furnished that iron probably is excreted by the glands. Concerning the fat and cholesterol content, it was noted that while all apocrine glands excrete small amounts of fat and cholesterol, by far the most excretion takes place in the axillary glands. No difference was seen in the histology or secretory activity of the apocrine glands in the two sexes. During pregnancy regressive changes and diminished secretory activity of the axillary glands were observed. Pathologic processes occasionally were encountered, such as cystic dilatation, atrophy with connective tissue proliferation, hyaline and amyloid degeneration of the basal membrane and epithelial proliferation. In the ceruminal glands of the ear concretions were frequently observed.

W. SAPHIR.

CLASSIFICATION OF NEPHROSIS. W. EHRLICH, *Virchows Arch. f. path. Anat.* **287**:333, 1932.

In five of six cases of glomerulonephritis associated with amyloid nephrosis, hyaline and fatty degeneration of the tubular epithelium was found. These degenerative changes are due not to circulatory disturbances of the glomeruli but more likely, as the author points out, to a primary toxic influence. Hence they must be regarded as a complicating primary nephrosis. Lipoid degeneration of the tubular epithelium was noted in all of the cases examined. Based on previous studies and his own work, the author proposes the following classification of nephrosis: (A) tubular nephrosis: (1) primary—cloudy swelling, hyaline and fatty degeneration, necrosis; (2) secondary—fatty infiltration, hemoglobin infiltration, biliary infiltration; (3) combined (primary and secondary)—lipoid nephrosis; (B) glomerulonephrosis, with or without lipoid degeneration—amyloid nephrosis.

W. SAPHIR.

THYROID GLAND OF NORTH GERMAN AND SOUTH GERMAN RATS. W. ORYWALL, *Virchows Arch. f. path. Anat.* **287**:348, 1932.

Endemic goiter is confined to certain localities and in such areas affects domestic animals as well as the human population. As a preliminary to a proposed study of the biologic reactions of the thyroid, the author made a study of the thyroid of white rats of Stuttgart, an endemic goiter region of south Germany, and of rats from Leipzig, a nongoitrous district of north Germany. The thyroid of the

Stuttgart rat, both absolutely and relatively to body weight, is considerably heavier than that of the Leipzig rat. The thyroid of the south German rat has relatively less colloid and more epithelial tissue, to which its greater weight is attributed. The thyroid gland of the Stuttgart rats responded to small doses of iodine by a marked loss of weight. This was due to an increase in the colloid content and a corresponding decrease of epithelial tissue. The thyroid of Leipzig rats responded to small doses of iodine in a similar way but to a less marked degree. Larger doses of iodine caused marked epithelial hyperplasia.

W. SAPHIR.

HISTOLOGY OF THE VALVULAR LESIONS OF ENDOCARDITIS LENTA. · R. H. JAFFÉ,  
Virchows Arch. f. path. Anat. **287**:379, 1932.

Jaffé presents the results of a histologic study of the valvular lesions of six hearts selected from among thirty-five cases of typical endocarditis lenta (Schottmüller) or subacute bacterial endocarditis (Libman) that came to necropsy at the Cook County Hospital, Chicago. In all of the cases selected, *Streptococcus viridans* was isolated from the blood. The duration of the disease varied from six week to eleven months, and the age of the patients, from 21 to 56 years. Special attention was directed to the early stages of the lesion, which are often best seen in areas not macroscopically involved. Infection of the valves occurs by way of the blood flowing over the surface of the valves, and only rarely by way of the coronary arterial circulation. The localization of bacteria leads to swelling, proliferation and necrosis of the endocardium and to a proliferative mesenchymal reaction of the subendocardial stroma. If, at this early stage, the tissue reaction is successful in restraining the bacteria, none of the latter are seen in the lesion, and the lesion resembles the verrucous lesion of rheumatic infection. If the bacteria are able to multiply, they are seen first in giant cells of fibrocytic origin. These cells undergo necrosis, and the characteristic clumps of bacteria are seen at the surface of the vegetation. The tissue about the clumps may early undergo calcification. The vegetation is formed, not of thrombotic material that is deposited on the surface of the injured valve, but of granulation tissue that has grown outward from the valve and that readily undergoes fibrinoid degeneration and necrosis. This productive, necrotizing, granulomatous lesion is characteristic of subacute endocarditis. It remains for future study to determine whether the lesion is histologically specific of infection by *Streptococcus viridans* or whether it may also be caused by some of the other species of bacteria that have been isolated from clinically typical subacute bacterial endocarditis. Should the latter prove to be the case, the valvular lesion would then be the morphologic expression of bacterial allergy.

O. T. SCHULTZ.

FIBROMYXOMATOUS HYPERPLASIA OF THE MITRAL VALVE. H. ENGEL, Virchows Arch. f. path. Anat. **287**:393, 1932.

A woman, 43 years of age, died suddenly with symptoms of cardiac failure. Necropsy revealed the mitral valve transformed into a polypoid, tumor-like formation of firm consistency. Histologically, the tumor was composed of fibromyxomatous connective tissue with numerous cell nuclei. In view of the absence of blood vessels and of inflammatory reaction, the author is inclined to classify the tumor in the group of endocardial tumors described by Ribbert, which are intermediate between hyperplasia and true blastoma.

W. SAPHIR.

THE HEART IN DEATHS DUE TO EMBOLISM AND THROMBOSIS. I. PUTNOKY and K. FARKAS, Virchows Arch. f. path. Anat. **287**:400, 1932.

In a series of 1,009 necropsies, death was due to embolism in 9 per cent and to thrombosis in 6 per cent. In 52 per cent of the patients who died of embolism and in 60 per cent of those who died of thrombosis, pathologic changes of the heart were found. In the cases of embolism, the changes were as follows: chronic



endocarditis, in 36 per cent; arteriosclerosis, 29 per cent; syphilitic aortitis, 17 per cent; acute endocarditis, 11 per cent, and myocardial fibrosis, 6 per cent. In the cases of thrombosis, the following cardiac conditions were found: arteriosclerosis, in 28 per cent; myocardial fibrosis and chronic endocarditis, 26 per cent, and acute endocarditis, 13 per cent.

W. SAPHIR.

VASCULAR CHANGES OF THE CENTRAL NERVOUS SYSTEM IN RHEUMATIC CHOREA. K. VON SÁNTHA, *Virchows Arch. f. path. Anat.* **287**:405, 1932.

In a girl, 15 years of age, who was suffering from chorea, complete muscular paralysis of all the extremities developed. She died sixteen days after the onset of the disease. Autopsy revealed, besides acute endocarditis and pneumonia, remarkable changes in the brain. The significant features were thrombosis, obliteration and recanalization of the small vessels of the pia mater. There were hyperemia, capillary endarteritis and small necrotic areas in the cortex and subcortex.

W. SAPHIR.

CARDIAC CHANGES IN DIPHTHERIA. DONNERSTAG, *Virchows Arch. f. path. Anat.* **287**:421, 1932.

The municipal hospital of Stettin has experienced an increasing number of deaths from diphtheria during recent years. The author reports the significant findings in a series of the fatal cases. In most of them death occurred with symptoms of cardiac failure shortly after the onset of the disease. Though gross cardiac changes were rarely noted, microscopically some or all of the following alterations were demonstrated in all cases: diffuse or circumscribed fatty degeneration, myolysis or hyaline degeneration of the muscle fibers, interstitial inflammation and connective tissue proliferation. The last two conditions were found only in the later stages of the disease.

W. SAPHIR.

CHANGES IN THE NERVOUS SYSTEM IN LEAD POISONING. H. FREIFELD, *Virchows Arch. f. path. Anat.* **287**:549, 1932.

Postmortem observations led the author to believe that there is no justifiable evidence for the assumption that lead is electively harmful to the nervous system. The harmful action is not due to a specific damage of ganglion cells, but is explained by the destructive action of lead on the blood vessels. Venous congestion and stasis interfere with the nutrition of nerve tissue and gradually lead to destruction of the tissue with the formation of glia. The genesis of lead colic is explained on the basis of degenerative changes of the solar plexus. Ingested lead is gradually absorbed by the impaired mucosa of the colon and taken up by the lymph, which carries it to the solar plexus. In the author's cases marked lymphangitis and dilatation of the lymph vessels of the retroperitoneal region were usually found.

W. SAPHIR.

EXTENSIVE NECROSIS OF THE INTESTINAL TRACT IN AGRANULOCYTOSIS. H. UFFENORDE, *Virchows Arch. f. path. Anat.* **287**:555, 1932.

In a case of agranulocytosis, the ulcerative lesions of the mucous membranes were not confined to the oral cavity, but were present in the entire alimentary tract from the oral cavity to the rectum. The most extensive changes were found in the esophagus, the mucous membrane of which was necrotic and separated entirely from the muscularis. The author presents a brief summary of the bibliography. Of 317 cases of agranulocytosis reported in the literature, the author believes that only 248 can be considered as true cases of agranulocytosis. Of these, there are only 40 (16 per cent) which offer proof for the assumption of an infectious etiology. In 37 cases the relation of preceding infection to the onset of the disease

seems doubtful. For the majority of the cases of agranulocytosis reported in the literature, the author considers as the most satisfactory explanation the assumption of an impaired myeloid system due to either congenital or acquired factors of various origin.

W. SAPHIR.

CHANGES IN THE OSSEOUS CAPSULE OF THE INTERNAL EAR IN RELATION TO THE HISTOPATHOLOGY OF THE SKELETAL SYSTEM. M. MEYER, *Virchows Arch. f. path. Anat.* **288**:1, 1933.

This is one of four articles on the pathology of the osseous system that occupy the first 211 pages of a number of *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*. Meyer's contribution, which interprets a variety of pathologic states of the skeletal system in the light of changes observed in the bony capsule of the internal ear, is based on the recent monograph of Nager and himself. In the skeleton under normal conditions, bone is constantly being resorbed and reformed; the skeleton is being continuously rebuilt. In diseases characterized by abnormal bone resorption or bone formation, what actually occurs may be difficult to determine because it is not always easy to distinguish between the normal and the abnormal processes. The development of the labyrinthine capsule is completed by the end of the second year of life. Thereafter no changes occur under normal conditions. Furthermore, the bone of the capsule has a different structure than that of the bone of the rest of the skeleton. It consists of islands of calcified embryonic cartilage embedded in compact bone that is fibrillated and not laminated like other compact bone. If the capsule participates in systemic skeletal diseases, the early completion of development and the different structure should aid in the interpretation of the general diseases, since resorption and new formation of bone can be readily detected and the newly formed bone is laminated bone and not the normal fibrillated bone. Pommer has claimed that in senile osteoporosis bone resorption is normal, but reformation of bone is deficient. According to Meyer, examination of the capsule of the internal ear establishes that new bone formation is very slight, but resorption of bone is marked. The moot question as to whether osteomalacia is primarily a regressive process Meyer answers by saying that the uncalcified bone is not original bone that has undergone regressive changes but is newly formed, uncalcified, laminated osteoid tissue that has been laid down on the trabeculae of the original fibrillated bone. He could detect no evidence of halisteresis in the labyrinthine capsule in osteomalacia. He agrees with Schmorl, Schmidt and others that rickets is essentially the same process as osteomalacia but differs in its effects because rickets occurs at an age when bone formation is active. In rickets little change was noted in the inner, enchondral portion of the capsule. But in the outer, perichondrial portion, the formation of osteoid tissue was so great and characteristic as to permit the histologic diagnosis of rickets. Fibrosis of the marrow is not the primary factor, but is secondary to the bony changes. Fibrosis of the marrow leads to deficient pneumatization of the mastoid. In osteodystrophia fibrosa, which includes Recklinghausen's disease and Paget's disease, the capsule of the internal ear reveals a characteristic mosaic structure identical with that of other bones. Osteoclastic resorption of the original bone is followed by the formation of immature bone, which becomes transformed into mature fibrillated bone. About the marrow spaces laminated bone is deposited. The mosaic pattern is due to the intermingling of the different kinds of bone in varying stages of maturity. The changes in the bony capsule of the internal ear were identical in Recklinghausen's disease and Paget's disease. More attention is paid to otosclerosis than to any other subdivision of the article. The changes in the bony capsule are identical with those of osteodystrophia fibrosa, of which disease otosclerosis is considered a form limited to the petrous bone. Identical histopathology does not necessarily presuppose a similar etiology. In endemic cretinism, the chief change in the temporal bone is hyperplasia and hypertrophy of the subperiosteal bone; the enchondral and perichondrial portions of the capsule of the internal ear reveal only slight change. In mongolian idiocy no changes were noted,

a fact that speaks against a postulated relation of mongolism to cretinism. Congenital osteogenesis imperfecta is characterized not only by disturbed osteoblastic function of the bone, but by a widespread disturbance of the mesenchyme. In chondrodystrophy, the cartilage of the labyrinthine capsule is invaded by strands of connective tissue carrying blood vessels.

O. T. SCHULTZ.

LYMPHOGRANULOMATOSIS OF BONE. E. UEHLINGER, *Virchows Arch. f. path. Anat.* **288**:36, 1933.

This report is based on the study of fifty cases of lymphogranulomatosis, in forty-eight of which the diagnosis was established by necropsy and in two by biopsy. The age of the patients varied from 12 to 77 years. Sixteen died in the third decade and twelve in the fourth. Twenty-three of the patients were males and twenty-seven females. Lymphogranulomatosis is a specific infection, which is histologically distinct from tuberculosis and lymphosarcoma. Primary involvement of bone or involvement limited to bone does not occur. Lymphogranulomatosis of the skeleton is a secondary process and arises either by direct continuity from a contiguous focus or by hematogenous metastasis. In Uehlinger's series, invasion of bone by direct continuity occurred twelve times and metastatic involvement five times. Under the metastatic cases Uehlinger includes only those in which the skeletal involvement was so widespread as to influence the clinical course of the disease. Direct erosive invasion occurred most often in the vertebrae, next in the sternum and pelvis and least often in the ribs, clavicle and scapula. The process begins in the periosteum, penetrates the lacunae of the cortex and spreads in the marrow, which is pushed aside or replaced. In the gross the process cannot be distinguished from an invasive neoplasm. The roentgenologic picture is not characteristic. Invasion of bone is usually painless and generally occurs relatively late in the course of the disease. Invasion of bone by direct continuity led to no characteristic or striking changes in the blood in Uehlinger's cases. In the marrow histologically specific tissue is formed; this tissue quickly undergoes fibrosis, even in the absence of irradiation therapy. The bone undergoes osteolysis and osteoporosis. The formation of endosteal and periosteal bone was infrequent. The radiosensitivity of the tissue varied and appeared to depend on the duration of the disease as a whole. The distribution of the lesions in hematogenous skeletal involvement coincided with the distribution of red marrow in the adult. The process begins as specific osteomyelitis, which leads to replacement and fibrosis of the marrow. Clinically, such cases were characterized by progressive anemia and leukopenia. The bone itself revealed relatively little change, and the roentgenologic findings were indefinite. The vertebrae, sternum and head of the femur were most often involved, the ribs, pelvis and cranium less often. Metastatic skeletal involvement occurred early in the course of the disease, which ran a rapidly progressive course.

O. T. SCHULTZ.

RENAL DWARFISM AND RENAL RICKETS. H. HAMPERL and K. WALLIS, *Virchows Arch. f. path. Anat.* **288**:119, 1933.

A detailed clinical and pathologic study of two cases of dwarfism is presented. Both patients were girls, aged 11 years and 3 months and 10 years and 5 months, respectively. The first had the stature of a girl of 6 years and 10 months, the second that of a girl of 5 years and 4 months. The body proportions were normal. The clinical findings were those of chronic interstitial nephritis, for whose onset no cause could be discerned in the history. The presence of chronic interstitial nephritis, with small contracted kidneys, was confirmed at necropsy. In the older girl, changes in the bones were very slight and were limited to the costochondral junctions of the ribs. The alterations were such as are seen in the ribs in very mild rickets. In the younger child, the roentgenologic and the histologic findings were those of marked rickets. The authors do not believe that the nephritis

was the direct cause of either the dwarfism or the rickets. If any important rôle is to be ascribed to the nephritis, it seems necessary to assume that the renal factor acts on a special constitutional factor. The authors prefer to assume that the same noxa caused the nephritis, the infantilism and the rickets.

O. T. SCHULTZ.

**SYPHILIS OF BONE.** E. FREUND, *Virchows Arch. f. path. Anat.* **288**:146, 1933.

Most of the literature of syphilis of the bone dates from a period when the histopathology of bone was not so well known. Freund's study, carried out in Erdheim's institute, is based on a study of the bones in five cases of late syphilis. The process begins as a gummatous osteomyelitis or as a syphilitic periosteitis, the agent reaching the tissues by way of terminal vessels, about which the first changes occur. Diffuse osteomyelitis occurs, but is rare. Diffuse productive periosteitis is more common, but the periosteal lesions may also be gummatous. Caseation may be followed by softening and removal of the necrotic material by phagocytic histiocytes, giant cells and leukocytes. If the removal is complete, the gumma is replaced by dense fibrous tissue. If the necrotic material is incompletely removed, it becomes encapsulated and may undergo calcification. As the inflammatory tissue invades the spongy bone, the latter may undergo lacunar resorption, leading to the formation of spaces into which the syphilitic tissue grows. If the invasion of bone is more rapid, the bone undergoes caseous necrosis. Porosis and sclerosis of bone occur about the gummas. Microfractures of the bony trabeculae are not uncommon. Larger fractures may lead to nearthroses. Healing may lead to a restitution of the osseous tissue to normal, to complete replacement of the shaft of a long bone or to the bony encapsulation of defects caused by necrosis.

O. T. SCHULTZ.

**UNUSUAL ANOMALY OF THE FACE IN AN ANENCEPHALIC MONSTER.** B. RATING, *Virchows Arch. f. path. Anat.* **288**:223, 1933.

In an anencephalic monster 32 cm. in length, an accessory nose was situated between a double right eye and the root of the normal median nose. The upper lip, palate and maxilla were cleft. The maldevelopment is made the basis of a discussion of incomplete reduplication anomalies of the face.

O. T. SCHULTZ.

**CONGENITAL ATRESIA OF THE LARYNX WITH HYPERTROPHY OF THE LUNGS AND GENERALIZED HYDROPS.** A. KOVÁCS, *Virchows Arch. f. path. Anat.* **288**:243, 1933.

In a female infant weighing 2,500 Gm., respiratory movements and contractions of the heart continued for twenty minutes after birth. The body was markedly edematous, and the peritoneal cavity was filled with fluid. Necropsy revealed complete atresia of the larynx and marked enlargement of the lungs. Other anomalies were absence of one kidney, ovary and oviduct; atresia of the anus and vagina, and ankyloblepharon. The increase in the size of the lungs was due to distention of the alveoli by mucus. The pulmonary hypertrophy and the hydrops were the direct results of the atresia of the larynx, which interfered with the escape of mucus from the bronchial system. Hydrops was the result of mechanical compression of the heart and vessels by the distended lungs. According to the author, only seven examples of congenital atresia of the larynx have been previously reported. In one of these the lungs were enlarged and there was hydrops. In other cases the state of the lungs was not noted. The author thinks that the enlargement was overlooked, since hydrops was present in several cases.

O. T. SCHULTZ.

ACCESSORY TONGUE. K. M. MENZEL, *Virchows Arch. f. path. Anat.* **288**:253, 1933.

Anomalies previously described as accessory tongue have been rounded masses in the floor of the mouth that contained cartilage or adipose tissue and that were attached to the body of the hyoid bone or to the septum of the tongue. They have had little structural resemblance to the normal tongue. Menzel carefully describes as accessory tongue two examples of a congenital anomaly that he claims has never been heretofore recorded. One was in a youth, aged 16, the other in a woman, aged 26. In each instance the accessory tongue was about the size of a walnut. It exhibited spontaneous fibrillary and coarser contractions and had a papillary surface like that of the base of the tongue. In the boy the mass was attached to the lower pole of the left tonsil. In the woman it was attached to the right side of the base of the tongue; the right tonsil and faucial pillars were absent. In each case the anomaly was associated with atresia of the external auditory canal, absence or maldevelopment of the external ear, maldevelopment of the middle ear and congenital paresis of the facial nerve, in each case on the same side as the lingual anomaly. The internal ear and labyrinth were functionally normal. Taste sensation of the accessory tongue of the boy was carefully tested and was found to be present and normal. Histologic examination of the structure after removal showed it to have a surface like that of the normal tongue, with filiform and circumvallate papillae and taste buds. The deeper portion of the mass consisted of striped muscle. Atresia auris associated with paresis of the facial nerve has frequently been described. These conditions are ascribed to defective development arising not later than the third fetal month in the region of the first and second branchial arches. Menzel believes that the accessory tongue described by him arose from multipotent tissue of this same region.

O. T. SCHULTZ.

ANOMALIES OF THE MÜLLERIAN DUCT. C. O. KESSELBURG, *Virchows Arch. f. path. Anat.* **288**:269, 1933.

Defective development of the derivatives of the müllerian duct is usually associated with cloacal defects. In the two fetuses described, the rectum and bladder were normal. In one, a rudimentary left uterus, to which a normal tube and ovary were attached, was connected with the bladder by a narrow duct whose opening into the bladder was situated beside the ureter of the same side. The urinary tract and a separate right uterus, tube and ovary were normal. In the second case there was complete duplication of the uterus. The left uterus, to which a tube and ovary were attached, opened into a normal vagina. The right uterus opened into a short, rudimentary vagina which communicated with the urethra and the left vagina. The right kidney, ureter, tube and ovary were absent.

O. T. SCHULTZ.

RELATION OF ALTERATIONS IN THE BRAIN TO HEPATIC DISEASE. H. J. SCHERER, *Virchows Arch. f. path. Anat.* **288**:333, 1933.

The constant occurrence of cirrhosis of the liver in Wilson's disease led to the histologic examination of the brain in forty-one cases of various kinds of hepatic disease. In addition to ordinary atrophic cirrhosis and acute yellow atrophy, the series included cases of pigmentary cirrhosis, fatty cirrhosis, widespread primary and secondary carcinoma of the liver and pseudocirrhosis of marked passive congestion. Changes in the macroglia were noted in 90 per cent of all the brains examined. These changes consisted in the presence of an increased number of enlarged, vesicular, naked nuclei. This process was independent of any parenchymatous changes in the brain. A probable histologic diagnosis of disease of the liver is possible from these cerebral changes. The degenerative changes of the ganglion cells of the basal ganglions, characteristic of Wilson's disease, were not observed in any instance in this series. The glial reaction of Wilson's disease is held to be secondary to the coexisting disease of the liver.

O. T. SCHULTZ.

CHANGES IN THE MUSCLES AND NERVES IN PARALYSIS AGITANS. ELSE PETRI, *Virchows Arch. f. path. Anat.* **288**:370, 1933.

Degenerative, regressive changes were noted in the brachial plexus and in the nerves of the forearm and in the muscles of the forearm, pharynx and tongue in a case of paralysis agitans which revealed relatively slight alterations in the central nervous system. The author suggests that death in this disease may be due to involvement of the diaphragm.

O. T. SCHULTZ.

CONTRACTED KIDNEY DUE TO SCLEROSIS OF THE MEDULLA. K. HELFAP, *Virchows Arch. f. path. Anat.* **288**:383, 1933.

The kidneys of 126 elderly persons were examined with reference to the degree of renal arteriosclerosis and the extent of fat and calcium infiltration of the medulla. No direct relationship between the two processes was found to exist. With the point of view of the independence of arteriosclerosis and medullary intertubular change as a background, the author describes four examples of marked fibrosis of the medulla which was associated with changes in the cortex and with relatively slight arteriosclerosis. The glomeruli were only slightly altered. The cortical involvement is held to be secondary to the medullary sclerosis and is likened to that of the hydronephrotic contracted kidney. Localized fatty infiltration and calcification of the medulla with tubular atrophy may lead to localized ascending sclerosis of the cortex. A combination of medullary sclerosis and arteriosclerosis may lead to the most extreme degree of renal contraction.

O. T. SCHULTZ.

### Immunology

QUANTITATIVE PRECIPITIN STUDY ON RABBITS INJECTED WITH AN AZO PROTEIN. M. HEIDELBERGER, F. E. KENDALL and C. M. SOO HOO, *J. Exper. Med.* **58**:137, 1933.

The preparation of a deep red protein dye, R-salt-azobenzidine-azocrystalline egg albumin is described, which contains no more than traces of protein with the original specificity of egg albumin. Based on previous publications of the authors, a method is given for the microscopic quantitative estimation of precipitin in antisera to the dye. The method gives the actual weight of the precipitin and may be applied to the determination of the maximum amount of precipitable antibody in any antiserum. Data are given (1) on the influence of the period between the final injection and the bleeding on the precipitin content of rabbit antisera to the azoprotein; (2) on the magnitude of the antibody response following the injection of multiple doses of the antigen varying within wide limits; (3) on the variations in the precipitin content of the sera of rabbits given successive courses of injections of the antigen, and (4) on the stability of the antisera in cold storage. Four antisera were obtained from which the amount of precipitin recovered was over one hundred times the amount of antigen injected. This supplements the growing mass of evidence against the theory that fragments of specific antigen are incorporated in the antibody molecule.

AUTHOR'S SUMMARY.

IMMUNIZATION WITH PURIFIED HAPTENS. H. MERCKENS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:308, 1933.

The purification of alcoholic extracts of organs, according to Weil and Ritzen-thaler, is based on removal of the lecithin and cephalin compounds with cadmium chloride and subsequent precipitation with acetone. Immune sera of rabbits treated with mixtures of such extracts and diluted hog serum possessed all the qualities of the sera produced with unmodified extracts.

I. DAVIDSOHN.

ADSORPTION AND ELUTION OF THE ANTIGENIC SUBSTANCE. A. J. WEIL, B. RITZENTHALER and H. MERCKENS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:316, 1933.

The antigenic substance can be removed from alcoholic extracts by adsorption with kaolin and other inorganic adsorbing compounds, and it can then be liberated by washing with water, physiologic solution of sodium chloride, alcohol or methyl alcohol, but not with acetone or ether. The adsorption is equally easy from acetone extracts, but the subsequent elution is not possible. The antigens which were purified according to the procedure of Weil and Ritzenthaler behaved in the same manner with regard to adsorption and elution as did the nonpurified extracts.

I. DAVIDSOHN.

MECHANISM OF THE FORMATION OF ANATOXIN. S. SCHMIDT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:339, 1933.

The article contains a review of the hypotheses by which authors have attempted to explain the nature of the detoxifying process. After a critical analysis of the available facts, Schmidt concludes that no satisfactory and fool-proof explanation has been advanced. The facts available are that the marked chemical and thermal resistance, the irreversibility of the detoxifying process and the drop of the amino-nitrogen values differentiate the anatoxin from the toxoid. Purified toxins are readily changed into anatoxins.

I. DAVIDSOHN.

TRANSFERABLE AND INHIBITING SUBSTANCES IN THE BLOOD OF ALLERGIC PATIENTS. W. KREMER, *Ztschr. f. Immunitätsforsch., u. exper. Therap.* **78**:382, 1933.

The transferable substances (allergens) were determined by means of the method of passive transfer, and the inhibiting substances (antiallergens) were demonstrated by mixing the allergens with the patient's blood or blood serum and then injecting the mixture intracutaneously. The usual cutaneous reaction was inhibited in the presence of antiallergens. Both types of substances are found frequently, but they need not be present simultaneously, as was seen in three patients who had only one kind of substance in the blood. Both types of substances always correspond with the allergens to which the patient is sensitive. They were never found in nonallergic persons. The antiallergens are transferable to the skin of other persons sensitive to the same allergens. The author concludes that the transferable allergens and the antiallergens are not identical.

I. DAVIDSOHN.

BOILED LIQUID SERUM AS ANTIGEN FOR THE PREPARATION OF PRECIPITATING ANTISERUMS. F. P. LEUSDEN and ERWIN PETRICH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:393, 1933.

Serums of the sheep, goat, ox, horse and pig and that of man were diluted with distilled water, boiled and used for immunization of rabbits. The precipitin titers for the homologous antigens and for the unheated serums were determined on several occasions during the treatment. The titers for the homologous antigens rose early, though only moderately, but soon became stationary, while those for the unheated serums rose slowly, reached a higher peak than the former and then dropped rapidly. Differentiation of the unheated and the boiled serum by means of precipitins produced with the boiled serum was not possible. Group reactions were observed with native and with boiled serums, while nonspecific reactions occurred only with boiled serums.

I. DAVIDSOHN.

INFLUENCE OF VENESECTION ON THE TITER OF DIPHTHERIA ANTITOXIN. E. F. GOGIN and E. G. ZURINOWA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:404, 1933.

The titer of the immune serum of horses was determined by means of the flocculation test of Glenn and Okell. The titer was highest eight days after the last injection of the antigen and dropped steadily afterward. The variations in

the titer could not be correlated with any known factors. The bleeding of the horses on the eighth and tenth days proved most satisfactory; an additional third bleeding is not advisable. The amount of serum obtained at the second bleeding was from 7 to 8 per cent larger than that obtained at the first.

I. DAVIDSOHN.

COMPARATIVE STUDIES OF THE PURIFIED TOXINS OF STREPTOCOCCI OF SCARLET FEVER AND OF ERYSIPELAS. W. A. KRESTOWNIKOWA and E. M. RJACHINA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:414, 1933.

The strains Dick 8 and Dochez were used for the preparation of the toxin of scarlet fever streptococci, and the strain Birkhaug 4c was the source of the erysipelas toxin. The purification consisted of removal of proteins, dialysis, inspissation, precipitation with alcohol and drying. The final product, a powder, was insoluble in lipid solvents and contained a carbohydrate substance. The two toxins differed in quantitative chemical composition; the difference became more or less obliterated with the progress of purification. Both products were toxic when injected intracutaneously, the toxicity of the scarlet fever streptococcus being very much more marked. Characteristic cutaneous and systemic changes were studied, the latter occurring only after intravenous introduction of the toxin of the scarlet fever streptococcus. Continued dialyzing lowered the toxicity of the toxin of scarlet fever to the level of that of the erysipelas toxin. Immune serum introduced one or two days previous to the intracutaneous injection prevented the appearance of the cutaneous manifestations. The toxin gave positive precipitation and complement-fixation reactions with immune serums. The antigenic action of the toxin of the scarlet fever streptococcus was much more pronounced than that of the erysipelas toxin. Potent immune serums were produced in rabbits treated with the purified toxins. The toxins possess all the qualities of full antigens.

I. DAVIDSOHN.

THE BEHAVIOR OF BRAIN EXTRACTS IN SEROLOGIC REACTIONS. GERTRUD PRÜSSE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:437, 1933.

The purpose of this investigation was to determine whether the frequently observed positive complement-fixation reactions between alcoholic extracts of brain and cerebrospinal fluid are specific or whether they are due to the composition of the fluid. The latter assumption was found to be true, because the fixation did not take place when normal human serum was added to the cerebrospinal fluid. The positive reaction of the fluid with the regular heart extract was only occasionally slightly weakened by the addition of normal serum. Not all human serums had the ability to modify the reaction of the cerebrospinal fluid. The reaction with brain extract of an antiserum against beef heart extract was similarly removed after the addition of normal serum. The addition of normal rabbit serum had a similar effect on the reaction between a heterophilic immune serum and a weak Forssman antigen, but the reaction with a strong antigenic extract was not influenced.

The author concludes: If cerebrospinal fluid giving a positive Wassermann reaction reacts with brain extracts and the blood serum does not react, the phenomenon is due not to the presence of specific brain antibodies in the fluid but to colloidal differences between cerebrospinal fluid and blood serum.

I. DAVIDSOHN.

MENINGOCOCCUS TOXIN. W. KRESTOWNIKOWA, A. BELKINA, E. DOSSER and I. LASOWSKY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:451, 1933.

Cultures of A, B and C strains of meningococci developed, after from five to six days, a toxic filtrable substance which was characterized by marked thermostability (it stood boiling for fifteen minutes) and by resistance to freezing and thawing. The toxin could be concentrated by Hunttoon's method. It was



toxic to mice, rabbits and guinea-pigs. Animals killed by intraperitoneal injections showed at postmortem examination pronounced toxic-degenerative, but nonspecific, changes. The changes in rabbits dying of meningitis after suboccipital introduction of the filtrate were similar to those which follow meningitis produced by bacteria. The filtrates reacted with immune and convalescent serums in the form of flocculation, precipitation and complement fixation. Mixing of bacterial immune serums with the filtrate protected mice, which shows that bacterial immune serums contain antitoxin. Formaldehyde detoxified the filtrate. Rabbits inoculated with the filtrate produced precipitating and complement-fixing immune serums. The toxic substance in the filtrates resembles the toxin of the scarlet fever streptococcus. It is a polysaccharid; it produces similar intracutaneous lesions in man and shows other similar features.

I. DAVIDSOHN.

SEROLOGIC SPECIFICITY OF THE SUPRARENAL MEDULLA. E. WITEBSKY and J. KLINKE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:509, 1933.

The serum of rabbits treated with suspensions of carefully separated medullary parenchyma of the suprarenal glands of oxen reacted in complement-fixation tests specifically with alcoholic extracts and less strongly with aqueous suspensions of suprarenal medulla of the ox, the hog and the sheep. Some antisera reacted slightly with suprarenal cortex, but the impression was gained that whenever the material which was used for immunization had been thoroughly freed from cortical parenchyma the organ specificity of the antiserum was absolute. Brain antisera reacted with extracts of suprarenal medulla, which is explained by the embryologic relations of both organs; however, antisera against suprarenal medulla failed to react with brain extracts. The results of this investigation increase the number of organs which are known to produce organ-specific immune serums to five (the lens, brain, spinal cord, posterior lobe of the hypophysis and suprarenal medulla).

I. DAVIDSOHN.

PROPHYLACTIC VACCINATION IN ICTEROHEMORRHAGIC SPIROCHETOSIS. HIDET-SUNE WANI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:1, 1933.

The spirochetes were grown in pure culture on a modification of Noguchi's ascitic fluid-kidney-paraffin medium. Inada and Ido are the authors of the modification. The vaccine was injected in two doses of 2 and 3 cc. between the shoulder blades. The vaccination was carried out on 10,268 miners who were employed in Japanese coal mines known to have been infested with the disease for many years. Only 0.13 per cent of those vaccinated contracted the disease, while among the untreated persons 1.12 per cent became infected. An epidemic in one of the mines was quickly terminated by vaccination of all miners. The immunity acquired by vaccination was present nineteen months after the treatment. The symptoms resulting from the vaccination were insignificant. The most serious complication was occasional formation of abscesses on the site of injection. An appendix contains a review of 14,686 vaccinations which were carried out by Majima in different groups of miners. The results were satisfactory.

I. DAVIDSOHN.

ADSORPTION OF ALCOHOLIC EXTRACTS OF ORGANS. TRUDE FISCHER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:39, 1933.

The article confirms the reports of various investigators that the haptens can be removed from alcoholic extracts of organs by means of various adsorbents. Cholesterolized alcoholic extracts of beef heart were deprived of their ability to fix complement in the presence of syphilitic serums after adsorption with barium sulphate and with calcium phosphate, but not after adsorption with aluminum hydroxide and kaolin. Such adsorbed extracts reacted with lipoid antiserum of rabbits. The heterophilic antigenic substance was removed both from undiluted and from diluted alcoholic extracts of horse kidney with specially prepared kaolin.

The kaolin was then able to react with a heterophilic immune serum. The antigenic substance could be removed from kaolin and calcium phosphate by treating them with alcohol, but treatment with distilled water and salt solution was not suitable for that purpose.

I. DAVIDSOHN.

SEPARATION OF LIPOID HAPTENS WITH INORGANIC COMPOUNDS. A. KLOPSTOCK and T. MISAWA. *Zuschr. f. Immunitätsforsch. u. exper. Therap.* 79:55, 1933.

The different haptens contained in alcoholic extracts of organs react differently with various adsorbing compounds. The investigation included the so-called lecithin hapten, which is present in all tissues and which reacts with serums produced in rabbits inoculated with commercial lecithin. Kaolin, aluminum hydroxide and calcium phosphate were not able to adsorb the lecithin hapten from alcoholic extracts of organs, but did absorb it from alcoholic solutions of commercial lecithin. The Wassermann lipid hapten appeared to be adsorbed from alcoholic extracts of heart when they were tested with human syphilitic serums, but the same extracts retained some ability to react with rabbit immune serums. The A group-specific hapten was removed by aluminum hydroxide and calcium phosphate from alcoholic extracts of organs and of blood; the species-specific hapten could not be removed. The ability of the organ extracts and of lecithin to activate the hemolytic ability of cobra poison was removed by treatment with kaolin, but the other compounds had little or no effect.

I. DAVIDSOHN.

IMMUNIZING ABILITY OF THE ADSORBED HETEROPHILIC HAPTEN. T. MISAWA. *Zuschr. f. Immunitätsforsch. u. exper. Therap.* 79:80, 1933.

The report deals with another attempt to throw light on the significance of the nonspecific protein which is necessary for successful immunization with the specific lipid hapten. The conception of Sachs, Klopstock and Weil that the protein is only an adjunct acting as a carrier would be supported if immunization were possible without admixture of an antigenic substance. Armangué and his associates claimed to have accomplished it by using kaolin, animal charcoal and other nonantigenic substances. Misawa failed to obtain an antibody response in rabbits treated with a mixture of an alcoholic extract of horse kidney with kaolin. A slight response was observed when animal charcoal was used as an admixture, but that result lost its significance when a rabbit which was inoculated with the charcoal only showed heterophilic antibodies. There may have been a connection between the peculiar antibody response and an infection of the inoculated animals with *Bacillus leptisepticus*, some strains of which have been shown to contain heterophilic antigen. The attempt to employ colloidium as the complementary antigenic admixture to the alcoholic extract of horse kidney failed, though in test tube experiments the colloidium adsorbed completely the Forssman antigen from the alcoholic extract.

I. DAVIDSOHN.

LOCAL ANAPHYLAXIS OF DIFFERENT ORGANS. HANS SCHOLER. *Zuschr. f. Immunitätsforsch. u. exper. Therap.* 79:99, 1933.

Rabbits were treated with subcutaneous injections of horse serum till they reacted with an Arthus phenomenon. The injection of horse serum into the wall of the small intestine or into the mesentery produced no inflammatory reactions, while distinct local reactions were noted when the injection was given into the wall of the stomach. The reaction consisted in edema, vascular stasis, perivascular leukocytic infiltration and early necrosis. No such reactions occurred following reinoculation with a heterologous serum. The results confirm the observations of Shapiro and Ivy in regard to the particular local sensitiveness of the gastric wall. The lack of reactivity on the part of the intestinal wall is not due to its inability to respond to local irritants.

I. DAVIDSOHN.

TITER AND CONCENTRATION OF PRECIPITINS. TAKEO SATOH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:117, 1933.

The titer of precipitins in an immune serum is a distinct and separate quality from their concentration. The concentration of the precipitins determines the content of the serum. The latter is determined by using the opposite procedure from that employed in the estimation of the titer: The immune serum is diluted and the antigen remains constant. Rabbits showed a high precipitin titer after a single injection, but the concentration of the antibodies was low; after repeated injections the concentration increased, while, as a rule, the titer remained approximately unchanged. The heterologous nonspecific precipitins were almost absent following a single injection; following multiple injections their titers were so high that the specific character of the serums was almost lost. The concentration of the heterologous precipitins was low, and the specific nature of the serum could be demonstrated by proper dilution with normal rabbit serum. The coarseness of the precipitate was greater in serums with a higher concentration of the antibody, but was not dependent on the titer. Resistance to heat, to chemicals and to physical influences and the complement-fixing ability of the immune serum were also dependent on the concentration of the precipitins, but not on the height of their titer.

I. DAVIDSOHN.

STANDARDIZATION OF ANTITOXIC STAPHYLOCOCCUS SERUM. H. GROSS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:163, 1933.

The hemolytic, the necrotizing and the systemic toxic qualities of the staphylococcus toxin are met by the corresponding antibody manifestations in the immune serums produced by treatment of rabbits with filtrates of from two to three week old broth cultures of strongly hemolytic and highly toxic staphylococci. Gross suggests the employment of the antihemolytic titer as an index of the antitoxic value of the immune serums. One-one hundredth cubic centimeter of a serum able to inhibit the minimal hemolytic dose of the toxin should be agreed on as the unit of antitoxin. The minimal hemolytic dose is the smallest amount of toxin which hemolyzes 1 cc. of a 1 per cent suspension of rabbit red blood cells after one hour of incubation at 37 C.

I. DAVIDSOHN.

ANTAGONISM BETWEEN RED BLOOD CELLS AND ORGANS OF THE RABBIT IN CONTENT OF A SUBSTANCE. WALTER TREIBMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:274, 1933.

The failure of rabbits without normal anti-A agglutinins to produce group-specific anti-A serums is due to the presence of the A quality in their tissues and blood serum. This was demonstrated by Treibmann by means of the inhibiting effect of such tissues on the hemolysis of sheep red cells by immune serums produced with human group A red cells. The procedure of the inhibition of hemolysis, as suggested by Brahn and Schiff, proved the most sensitive of all methods for the demonstration of the quality A. Complement fixation with watery suspensions and with alcoholic extracts was also employed. The substance A could not be demonstrated in the red blood cells of rabbits which had varying quantities of it in the other tissues. Rabbits with normal anti-A agglutinins in their serum do not possess the A quality in their tissues.

I. DAVIDSOHN.

ANALYSIS OF BLOOD ANTIGENS WITH THE HELP OF HETERO-AGGLUTININS. M. EISLER and A. HOWARD, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:293, 1933.

The effect of red blood cells of different species of animals on normal hetero-agglutinins in their serums permitted the recognition of identical antigenic substances in the blood cells of distant species and of differences in the blood cells of closely related species and of individuals of the same species. The results con-

firmed those obtained by various authors in the study of immune serums against various bloods. The distribution of the common antigenic substances shows no apparent relation to the zoological system. Witte's peptone inhibited the agglutination of certain red blood cells by some normal serums. The inhibiting effect of the peptone was removed by fecal extracts.

I. DAVIDSOHN.

PRODUCTION OF ANTIBODIES AGAINST CARBOHYDRATES (ACACIA). P. UHLENHUTH and E. REMY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:318, 1933.

As far back as 1904, Uhlenhuth reported flocculation in a mixture of a suspension of acacia with normal serum of some animals. In rabbit serum which did not react with flocculation normally, flocculation occurred after immunization with acacia. The in vitro reaction also took place with arabic acid and with its calcium salt. Uhlenhuth in the original publication emphasized the significance of the observation, which suggested antigenic qualities of a carbohydrate substance, but reserved a definite opinion till arabic acid should be produced in a chemically pure state. The experiments were recently successfully repeated with the cooperation of Remy. The production of antibodies was demonstrated by means of the precipitation and complement-fixation reactions. The failure to obtain a nitrogen-free arabic acid again prohibited definite chemical conclusions as to the antigenic rôle of the carbohydrate in acacia.

I. DAVIDSOHN.

SEROLOGIC SPECIFICITY OF THE PINEAL GLAND. E. WITEBSKY and H. REICHNER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:335, 1933.

Relative and quantitatively limited serologic specificity of the pineal gland could be demonstrated by means of immune serums produced in rabbits with aqueous suspensions of the organs, while immune serums produced with alcoholic extracts did not permit differentiation from the brain. Differentiation from other organs than the brain was accomplished. The organ-specific quality is therefore insoluble in alcohol and thermolabile.

I. DAVIDSOHN.

BEHAVIOR OF COMPLEMENT AND OF ITS COMPONENTS DURING ADSORPTION AND ELUTION. LEO OLITZKI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:347, 1933.

For adsorption, kaolin, aluminum hydroxide and a compound, *silargèle* (the nature of which is not mentioned), were used. The attempts to liberate the adsorbed active substance by washing with various solutions (glycine and sodium chloride, glycine and sodium hydroxide, sodium phosphate and glycerin) were not successful when the whole complement was adsorbed, but some of the active principle was freed when the separated midportion was used. The end-portion of the complement had a greater activating ability than the midportion.

I. DAVIDSOHN.

THE CONTENT OF AGGLUTININS IN COMBINED ORAL AND SUBCUTANEOUS TYPHOID IMMUNIZATION. HEINRICH RUGE, *Zentralbl. f. Bakt. (Abt. 1)* **124**:276, 1932.

Twenty-two men were given a lysate of typhoid bacilli by mouth and four weeks later were immunized subcutaneously with the same type of lysate. Agglutination tests made four weeks after the oral administration of the lysate were positive in two thirds of the subjects. Following subcutaneous vaccination the tests showed higher titers in those who had previously given positive reactions after oral immunization than in those who had received the lysate only subcutaneously. The author concludes that oral immunization favors the formation of agglutinins.

PAUL R. CANNON.

## Tumors

GLIOMA OF THE CEREBRAL HEMISPHERES. H. BERGSTRAND, *Virchows Arch. f. path. Anat.* **287**:797, 1933.

The author examined about one hundred cerebral gliomas. One third of them were clinically benign, and two thirds were clinically malignant. Following a detailed description of the specimens, the author considers Cushing and Bailey's classification too artificial and furthermore unnecessary. He proposes a subdivision into benign astrocytoma and malignant glioblastoma. At times, however, it may be impossible to draw a sharp line between these two forms.

W. SAPHIR.

MORPHOLOGY OF GLIOMA. L. SINGER and J. SEILER, *Virchows Arch. f. path. Anat.* **287**:823, 1933.

The authors' studies lead them to conclude that the astrocytic glia of the glioma must be differentiated from the tumor parenchyma. The astrocytic glia and the mesodermal reticulum constitute the stroma of the growth. The tumor tissue itself is held to originate in differentiated nerve cells. According to this conception, the name "glioma" is misleading and should be abolished. The authors reject Cushing and Bailey's classification and propose a simple classification into medulloblastoma and ganglioneuroma according to the nature of the specific tumor cells. The character of the stroma may be indicated by the adjectives "reticular," "syncytial" and "neuromatous."

W. SAPHIR.

NEOPLASTIC RUPTURE OF THE SPLEEN. M. PLONSKIER, *Virchows Arch. f. path. Anat.* **289**:370, 1933.

A man, aged 63, died of intraperitoneal hemorrhage due to spontaneous rupture of the spleen. Examination during life had brought to light marked anemia and leukocytosis (white cell count, 34,000), most of the cells being myelocytes. At necropsy a mass in the mediastinum was held to be a primary tumor. There were numerous metastases in all the organs, including the spleen, which weighed 540 Gm. and had ruptured. Histologically the tumor was suggestive of carcinoma in places, but its exact origin remained in doubt, although it was held to be probably a reticulo-endothelial sarcoma.

O. T. SCHULTZ.

LIPIDS OF TUMORS OF THE SKIN. L. SZODORAY and P. SPANYÁR, *Virchows Arch. f. path. Anat.* **289**:493, 1933.

Thirty-one tumors of various kinds were examined by the various histochemical methods for fats and lipoid substances. In twenty-one of these tumors, which included seventeen tumors of the skin, quantitative chemical estimation of total fat, neutral fat, lipoids and cholesterol was also carried out. The neoplasms of the skin contained a relatively large amount of visible fat, which was not necessarily associated with an increase in total fat. The histochemical methods are not sufficiently specific to permit identification of the various kinds of fatty substances that may be present within the cells. The most striking finding in the tumors of the skin was an increase in cholesterol, which constituted from 5 to 21 per cent of the total fatty substances and more than 1 per cent of the dry weight of the tumor tissue.

O. T. SCHULTZ.

CALCIFYING EPITHELIOMA OF THE SKIN. W. FINK, *Virchows Arch. f. path. Anat.* **289**:527, 1933.

Calcifying epithelioma of the skin has been held by many to be a characteristic kind of epithelioma. In 1930 B. Fischer described calcification of atheromatous epidermal cysts, which raised the question whether all of the so-called calcifying

epitheliomas of the skin are true epitheliomas. Fink examined histologically nine such tumors and a number of atheromatous cysts. He concludes that the term calcifying epithelioma includes two kinds of structures. Some are true basal cell epitheliomas in which the epithelial parenchyma has undergone degeneration and fatty change. This is followed by connective tissue organization and calcification of the degenerated material, and sometimes by true bone formation. Others are atheromatous epidermal cysts in which inflammation of the capsule has led to penetration of organizing connective tissue into the fatty contents of the cyst and to calcification. If the epithelium has been completely degenerated it is impossible to decide the origin of the calcified tumor. Although the so-called calcifying epitheliomas may be either true epitheliomas or atheromatous cysts that have undergone calcification, the process is identical in both and is essentially one of organization of degenerated epithelium.

O. T. SCHULTZ.

BRANCHIOGENIC CARCINOMA IN A HORSE. P. KOSTER, *Virchows Arch. f. path. Anat.* 289:544, 1933.

Although branchiogenic carcinoma of the neck is not uncommon in human beings, its occurrence in domestic animals has been described infrequently. The author describes a typical branchiogenic squamous cell carcinoma in a horse.

O. T. SCHULTZ.

COLLISION CARCINOMA AND SARCOMA. W. NOWICKI, *Virchows Arch. f. path. Anat.* 289:564, 1933.

The author describes two neoplasms composed of an admixture of carcinomatous and sarcomatous tissue. One was a tumor of the mammary gland of a woman aged 45. In its peripheral portion it was pure glandular carcinoma. Centrally it was spindle cell sarcoma with numerous large, multinucleated giant cells. In an intermediate zone the two kinds of tissue were intermingled. The second tumor was a nodule that apparently arose from the wall of a bronchus. At the pole which had replaced bronchial tissues it was a bronchogenic carcinoma. At the opposite pole it consisted of spindle cell sarcoma. The midportion was composed of an admixture of the two kinds of tissue. The author interprets both tumors as the result of the independent development of carcinoma and sarcoma and of the invasion of one type of neoplastic tissue by the other where the two kinds came in contact. He discusses the nomenclature of tumors composed of both carcinomatous and sarcomatous tissue. For those that develop as two distinct types of neoplasm which become intermingled in the course of their growth he prefers the name collision carcinoma and sarcoma.

O. T. SCHULTZ.

ONCOCYTES IN SALIVARY GLANDS AND THEIR RELATION TO TUMORS. G. STEINHARDT, *Virchows Arch. f. path. Anat.* 289:624, 1933.

Steinhardt's report is based on histologic examination of salivary glands in sixty-five necropsies, undertaken with the view of determining the correctness of Hamperl's assertion (*Virchows Arch. f. path. Anat.* 282:724, 1933; abstr., *ARCH. PATH.* 15:735, 1933) that tumors of the salivary glands and lympho-epitheliomas of the buccal cavity and pharynx may arise from cells that he termed oncocytes. These are derived from glandular and duct epithelium by a process which Hamperl termed senile differentiation. Steinhardt could find no oncocytes in any of his material, although he did find what Hamperl had described as a transitional form in eight cases, all occurring in persons over 60 years of age. Steinhardt agrees that these are senile cells. Nodular and papillary hyperplasias of such transitional cells were seen a few times. He does not agree that lympho-epitheliomas are derived from such cells or from oncocytes, but considers that they probably arise from branchial cleft rests. He describes a solid adenoma of the parotid gland.

O. T. SCHULTZ.

TUMORS OF THE FOURTH CEREBRAL VENTRICLE. B. KELLNER, *Virchows Arch. f. path. Anat.* **289**:656, 1933.

Two tumors of the fourth ventricle are described. One is considered an ependymal glioma; the other, an epithelioma derived from the covering cells of the choroid plexus.

O. T. SCHULTZ.

PRESENCE OF RECEPTORS M AND N IN TUMORS. ALFRED ZACHO, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **77**:520, 1932.

The substances M and N were found in thirteen malignant tumors; they were absent in seven benign growths. The determination was based on absorption of purified immune serums containing the substances M and N by aqueous extracts of tumor tissues. Complement fixation was found unsuitable for the detection of substances M and N.

I. DAVIDSOHN.

SEROLOGIC ANALYSIS OF SARCOMATOUS TISSUE. E. WITEBSKY and E. MORELLI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:179, 1933.

The serum of rabbits treated with sarcomatous tissue reacted in complement-fixation tests most strongly with sarcoma extract, less with carcinoma extract, least with normal tissue extract and not at all with extract of rat or chicken sarcoma. The A group-specific quality could not be demonstrated in the sarcomatous tissue, although it was regularly present in the carcinomatous tissues of the same blood group. The reactivity of the immune serum was not due to a larger amount of lipoids in the sarcoma as compared with the normal organs. Adsorption of the immune serum with alcoholic extracts of normal organs removed the ability to react with those organs but left the ability to react with carcinomatous and sarcomatous extracts. Adsorption with carcinomatous extracts left only the reactivity with sarcomatous tissues. All antibody functions were removed by adsorption with sarcomatous extracts. The relation between sarcomatous and carcinomatous antigenic functions appears closer than that between the antigenic functions of sarcomatous and normal organs, with the exception of the spleen. The quality of the sarcoma immune serum depends more on the properties of the antigen than on the animal used for the injection.

I. DAVIDSOHN.

DEMONSTRATION OF ANTIBODIES AGAINST MALIGNANT TUMORS IN BLOOD. HANS J. FUCHS, M. V. FALKENHAUSEN and W. K. DEVRIENT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **78**:272, 1933.

Fuchs has worked out a test for malignancy which is based on determination of the nonprotein nitrogen in a mixture of the fibrin or of a specially prepared precipitate of the serum of patients with malignant tumors with the serum of normal persons, and vice versa. The fibrin and serum of affected persons may contain an antigenic substance specific for malignant growths, or antibodies directed against the tumor antigen. The presence or absence of such antibodies is determined by the phase of the disease and by the immunity responses of the body. Thus, a mixture of the serums of two carcinomatous patients may show a decrease of the nonprotein nitrogen, indicating a positive reaction, if the patients are at the time in different phases of the disease, or no such change if both are in the same phase. The method can also be used for the study of other diseases in which antibodies are produced.

I. DAVIDSOHN.

MALIGNANT GIANT CELL TUMOR OF BONE. W. I. KORCHOW, *Ztschr. f. Krebsforsch.* **38**:380, 1933.

The author gives a detailed description of a case of so-called giant cell sarcoma of the tibia, with ultimate death. There were numerous metastases, both local and generalized. In these metastases there was complete absence of the giant

cells, except in some of the tumors in the skin close to the original growth. The structure was that of malignant development of the tumor stroma. Korchow, in common with most recent writers on these tumors, regards the primary giant cell growth as dystrophic rather than neoplastic, with, in such cases as that discussed here, a secondary and superposed malignant alteration.

H. E. EGGERS.

CARCINOMA OF THE ALIMENTARY TRACT AT EARLY AGES. G. WOLFF, *Ztschr. f. Krebsforsch.* 38:409, 1933.

Wolff bases his rather elaborate study of the incidence and course of carcinoma of the alimentary tract at relatively early ages both on cases seen at the clinic of Freiburg University and on those reported in the literature. He finds that precocious cancers occur with special frequency in this location (in about 69 per cent of cases). The upper part of the tract, from the mouth to the stomach, is rarely involved primarily. The growths occur in the stomach with frequency, about 25 per cent of such carcinomas being primary here; they originate in the intestine, exclusive of the rectum, in from 10 to 15 per cent of the cases, and in the rectum the frequency of primary involvement is about the same as in the stomach, or 25 per cent. As to the accessory organs, primary cancer of the liver, always rare, occurs with rather surprising frequency in young persons, about 4.5 per cent of all carcinomas in children originating here. Precocious cancer is rare in the gallbladder and pancreas. There are no apparent differences in sex incidence, nor do such cancers show any definite peculiarities of course, except that in Wolff's experience the course appears to be somewhat accelerated. The etiology is discussed principally in terms of Virchow's irritation theory versus that of displacement as advocated by Cohnheim and Ribbert.

H. E. EGGERS.

RELIABILITY OF STATISTICS ON CANCER. A. E. SITSSEN, *Ztschr. f. Krebsforsch.* 38:639, 1933.

In this critical discussion of the value of statistics on cancer, Sitsen divides them into three classes as regards source: (1) those drawn from autopsy reports of various pathologic institutions, (2) those from mortality reports, and (3) those from reports based on clinical findings. As regards the first, their evidence is as a rule accurate, but their availability for general conclusions is affected by the limited character of the clientele of the institutions and by the fact that, particularly in the charitable institutions, the types of cases vary with the interests of the responsible members of the staffs and with the general effectiveness of treatment, since with fewer deaths from other diseases those from cancer will show a corresponding increase. Statistics on mortality are of general application but are open to the patent objection of questionable diagnosis—a factor which cannot be evaluated even approximately. With statistics from clinical sources there is even greater liability to error through inaccurate diagnosis, but they have the great advantage of giving information as to current cases rather than as to those that have terminated in death. In Austria a systematic effort is being made to collect information of this sort comprehensively and without reduplication. No statistics on cancer from a single source are satisfactorily accurate, but the errors lie not in the methods of collection but in their execution. Ideal use of all three sources is attainable, as is indicated in the collection of statistics in the Dutch East Indies by Snijder and Straub; they have, for their district, reports of complete physical examinations of the entire population, and over 90 per cent of patients who die come to autopsy. Such results can be achieved generally only by education of the populace to the fundamental importance of routine autopsies.

H. E. EGGERS.

WHAT SHOULD BE MEANT BY THE TERM "PRECANCER"? H. T. DEELMANN, *Ztschr. f. Krebsforsch.* 38:648, 1933.

The term "precancer," widely used by clinicians, is according to Deelmann objectionable since it is used to include conditions in which cancer is only a probability or even only a possibility. To the pathologist the word should convey



a definite significance—that of the local “unrest” of cellular elements which so frequently precedes the infiltrative growth that constitutes developed cancer. Changes of this sort are not infrequently seen most typically at the margins of carcinomas and appear there to indicate a radial transmission of cancerous activity to cells originally not involved. Disordered and evidently excessive epithelial growth of this type, as yet without actual infiltration, certainly is not cancer, but as indicating a change immediately precedent to infiltrative growth it is precancerous in the strictest sense.

H. E. EGGERS.

### Medicolegal Pathology

TESTS FOR M AND N BLOOD GROUPS. W. CROME, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **21**:435, 1933.

The tests should be made by persons skilled in serologic technic. The results of 3,800 serologic observations are recorded. The simultaneous absence of M and N factors was never observed. Of the entire series, 19.6 per cent belonged to group N, 30.8 per cent to group M and 49.6 per cent to group M N. According to the four group scheme, there were 39.7 per cent in group O, 47.7 per cent in group A, 9.6 per cent in group B and 3.5 per cent in group A B. A study of heredity in 32 families with 82 children showed that the mendelian laws were strictly followed. Four pairs of monozygotic twins had the same blood group. In a series of 46 paternity tests, 3 persons were excluded as possible fathers on the basis of the M and N groupings.

JACOB KLEIN.

PENTAMETHYLENETETRAZOL POISONING. A. ESSER and A. KÜHN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **21**:474, 1933.

A 22 year old man died one hour after drinking 100 cc. of a 10 per cent solution of pentamethylenetetrazol with suicidal intent. Clinically there was observed a state of excitation and convulsions simulating jacksonian epilepsy. Macroscopically the viscera appeared normal. Microscopically there were marked hyperemia of all the organs, cloudy swelling of the liver and kidneys, a great decrease in the suprarenal lipoids, fatty changes in the liver and fragmentation and fatty changes in the myocardium. Animals experimentally poisoned showed similar changes. Pentamethylenetetrazol could be demonstrated in all parts of the body and in its fluids. One third of the ingested amount was recovered from the tissues. Experiments demonstrated that this substance could be recognized even in putrefying organs. The drug is absorbed chiefly from the stomach and excreted mainly through the kidneys. The pharmacologic views as to the fleeting action of pentamethylenetetrazol do not hold for the toxic dose. Animals receiving slightly toxic doses have shown effects after twenty-four hours. Some have apparently recovered only to die after several days. It is estimated that the minimum lethal dose is 7.8 Gm. of pure pentamethylenetetrazol for an adult human being weighing 65 Kg.

JACOB KLEIN.

CHARACTERISTIC SKIN MARKINGS AFTER FALLING INTO THE WATER FROM A HEIGHT. GYULA BALAZS, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **21**:515, 1933.

Persons who have jumped into the water from a bridge (10 meters high) and have been saved have shown characteristic anemic areas on the skin in the area which hit the surface of the water. These anemic regions, and even hematomas, occur most commonly over the thigh and gluteal region, particularly over the femur and the obturator foramen, and result from pressure of the tissues against the bony framework. After twenty-four hours the cutaneous changes begin to diminish, and they disappear after from three to four days unless there is a hematoma. The markings have been seen in 15 of 250 cases. They indicate a vital reaction on falling into water from a height and may give an indication as to the position of the body when it hit the water.

JACOB KLEIN.

# Society Transactions

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## PATHOLOGICAL SOCIETY OF EASTERN NEW YORK

ARTHUR W. WRIGHT, *Secretary*

*Regular Meeting, April 7, 1933*

F. F. HARRISON, *Presiding*

### FATAL ERYTHROBLASTIC ANEMIA. V. C. JACOBSEN.

An Italian boy, 3 years of age, had had enlargement of the spleen and liver for two years. The spleen filled the entire left side of the abdomen and extended down into the pelvis. There was weakness. The hemoglobin was 40 per cent: the erythrocyte count, 2,800,000; the leukocyte count, 22,600, with 73 per cent polymorphonuclears, 20 per cent lymphocytes and 7 per cent myelocytes. Fifty nucleated red blood cells were seen. There was slight persistent fever. The treatment consisted of blood transfusion, application of 800 milligram-hours of radium and later administration of liver extract no. 55.

After eight months the spleen was still greatly enlarged. The hemoglobin was 28 per cent. The erythrocyte count was 2,400,000; the leukocyte count, 16,600, with 25 per cent polymorphonuclears, 2 per cent basophils, 40 per cent lymphocytes, 17 per cent monocytes and 16 per cent myelocytes. Sixteen nucleated red blood cells and 30 reticulocytes were seen. Liver extract was given intravenously, without improvement. Death occurred from a respiratory infection.

At necropsy, the abdomen was distended and prominent while the remainder of the body showed marked emaciation. The mesenteric lymph nodes were large and pale. The spleen weighed 450 Gm. and occupied the entire left side of the abdomen. The surface showed hyaline capsulitis. The organ was firm, rather rectangular and brickish red. Sections showed a firm, grayish-red, bulging, finely granular pulp. The lymphoid tissue was indistinct. The liver weighed 940 Gm. and was pale chocolate-brown. Section revealed a pale parenchyma containing numerous small foci of necrosis. The kidneys were of normal size and extremely pale except for a few petechial hemorrhages. The muscles were pale grayish red and thin. The bone marrow of the ribs was small in amount and pale. The vertebral marrow was dry and congested, with a few pale areas scattered throughout. The marrow of the ilium was congested and apparently more cellular than usual.

Microscopic examination of the spleen showed obliteration of many sinuses and atrophy of the follicles. Many foci of mononuclear cells of myeloblast and erythroblast types were present with eosinophilic myelocytes and mature polymorphonuclears in small numbers. Megakaryocytes were numerous, as well as large cells resembling the Dorothy Reed cells of Hodgkin's disease. Very little hemosiderin was found. In the liver, foci of acute hyaline necrosis with invasion by polymorphonuclears were present. Much hemosiderin was found in both hepatic and Kupffer cells. The kidneys were edematous, and there was an increased amount of lipoids in the cortical tubules. The bone marrow from whatever location was very cellular, with marked hyperplasia of the erythrogenic centers. Many myeloblasts and erythroblasts of large and small types were present, as were polymorphonuclears of all stages and many eosinophils and megakaryocytes. There was no phagocytosis of erythrocytes, and hemosiderin was not observed. Bone trabeculae were abundant and normal.

The clinical and postmortem observations were typical of a type of blood dyscrasia seen in childhood and known as erythroblastic anemia. Many of the cases reported have occurred in the Mediterranean races, as did this case.

## SARCOMA OF THE CHOROID. STEPHEN H. CURTIS.

A woman, 62 years of age, first noticed in October, 1931, that the sight of her right eye was failing. Gradually she began to suffer severe pain in this eye, the pain being more severe at night. The conjunctiva of the eye was injected and the orbit protruded slightly.

Physical examination of the eye showed moderate exophthalmos, mild acute conjunctivitis, increased intra-ocular tension and paralysis of the pupil. The ophthalmoscope revealed a dark red mass protruding from the retina medial to the optic nerve. The retina about the tumor was detached.

The eye was enucleated in January, 1932. Externally it showed nothing of note. On section a firm, pedunculated, cauliflower-like mass, 1 cm. in diameter, was found projecting from the retina near but not involving the optic nerve. Cut surfaces of the mass were pearly gray, smooth and glistening.

Microscopically, the tumor was composed of two types of cells, which were probably variants of a parent cell. One type was long, spindle-shaped and afibrillar, with oval, pale-staining nuclei. The other was large, round or polyhedral, with abundant cytoplasm and large, pale, often cystic nuclei. Moderate numbers of the cells of both types contained melanin. No neurofibrils or rosettes could be found. Mitoses were absent. Stroma was not abundant.

This tumor presented the characteristics of a nonpigmented or at most very slightly pigmented melanosarcoma of the choroid. By some pathologists, however, the tumor was thought to be a neurogenic sarcoma of the retina. The evidence, both clinical and pathologic, favors the former diagnosis.

## UNUSUAL LIPOID CHANGE IN AN OVARIAN FIBROMA. ELLIS KELLERT.

A woman, 43 years old, unmarried, had had pelvic pain, backache and abdominal enlargement for nine months. Both the tubes and the ovaries were removed. One tube was attached to a parovarian cyst 7.5 by 5 by 3 cm. in size. The opposite ovary was replaced by a tumor 13 cm. in diameter. The serosa was smooth, gray and traversed by many prominent blood vessels. On section there was found a yellowish, solid tumor containing a single large and several small cavities, which were empty and collapsed. To their linings were attached shaggy fibrinous masses of clot and a small amount of grayish-yellow amorphous material. The thick, solid portion of the growth was at the attached margin of the ovary. Its cut surface was rough, fibrous and bright yellow.

Microscopic sections showed interlacing bands of spindle-shaped cells containing short, oval, deeply staining nuclei and resembling ovarian stroma. There were no mitoses. The intercellular substance was variable in amount and, where abundant, was associated with an enormous number of fat globules, which varied greatly in size and were found both within and between the cells. There were no distinct adipose cells. In sections where the fat had been dissolved with chloroform, only a cribriform connective tissue network containing many of the oval nuclei was seen.

On prolonged immersion of the tissue in solution of formaldehyde the yellow color faded and became gray. The changes observed were probably degenerative and were perhaps due to circulatory disturbances.

## MENINGIOMA OF THE LEFT CEREBRAL HEMISPHERE. ELLIS KELLERT.

A woman, aged 40, had had severe intermittent headaches and occasional convulsions for four years. Recently jacksonian epilepsy developed with twitching of the right side of the body and of the face. The right hand was weak, and sight was poor in the right eye. When speaking, the patient would frequently hesitate because she could not find the right word. A clinical diagnosis of tumor of the right hemisphere was made.

The brain weighed 1,440 Gm. In the left frontoparietal region there was a globular tumor, 7 cm. in diameter, attached to the dura and pushing into the brain. During the handling of the brain the tumor enucleated spontaneously. It was

encapsulated and on section appeared grayish white and solid with granular cut surfaces. Sections showed a very cellular growth consisting of spindle-shaped cells of fairly uniform size, having a whorl-like arrangement. Mitotic figures were not found. A diagnosis of meningioma was made.

#### A MIXED TUMOR OF THE URINARY BLADDER. G. H. KLINCK, JR.

A white girl, 16 years of age, had been having increasing difficulty in voiding for several months. She discovered that if she inserted her finger into the urethra she could void. Cystoscopic examination showed a soft, pedunculated tumor, the size of an orange, attached just within the orifice of the right ureter. The tumor was removed by suprapubic cystotomy. It was a soft, globular mass about 7 cm. in diameter. The surface was slightly uneven, some areas being smooth and pale, others slightly roughened and congested. There was a very delicate and easily broken capsule. The pedicle measured 6 mm. in length and 3 mm. in diameter. Section revealed soft, myxomatous, semitranslucent tissue with slightly opaque foci. Microscopically, the tissue was largely myxoma but with coarse strands of fibrous tissue. Sections from the surface showed acute inflammation. A diagnosis of fibromyxoma was made.

The patient was entirely well for a year; then hematuria developed. A tumor was found within the bladder at the site of the suprapubic incision. It was irregularly distributed along the line of the old incision. This area, including the tumor, was removed, the mass being approximately 30 cc. in volume. Grossly, the tissue resembled the original tumor. Nine hundred and fifty milligram-hours of radium were applied to the edges of the new suprapubic incision before closing the wound.

Microscopic study showed a myxomatous growth like that found previously but containing, in addition, small islands of cartilage and numerous striated and smooth muscle fibers. It is probable that both cartilage and muscle were present in the original tumor but were not found in the sections made.

Two months after the second operation, a recurrence appeared in the healed wound of the cystotomy. The right ureter was normal. When the patient was last seen, six months after the final operation, there was an increase in the size of the midline growth. A biopsy showed all of the features previously described.

The tumor was a teratoma, although only mesodermic elements were present, namely, striated and smooth muscle, myxomatous connective tissue and cartilage. It was not histologically malignant, but recurrences developed probably on the basis of cells left behind at the various operations and also seeded along the operative incisions.

## Book Reviews

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**Wilhelm Conrad Röntgen and the Early History of the Roentgen Ray.** By Otto Glasser, Ph.D., Director, Radiation Research Department, Cleveland Clinic. With a chapter on personal reminiscences of W. C. Röntgen, by Margaret Boveri (Berlin). Price, \$6. Pp. 496, with 96 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

This book is a translation with corrections and revisions of the original German edition of 1931, which was well received. It is the product of the cooperation of three publishers, Julius Springer of Berlin, Charles C. Thomas of Springfield, Ill., and John Bale, Sons and Danielsson, Ltd., of London. The book is printed by the last named firm. The paper is heavy and highly glazed, which is unfortunate. The first four chapters describe vividly the details of the discovery in 1895 of the roentgen rays, the circumstances of its announcement and the first reception. The biographic interest of the book centers in the fifth chapter (147 pages). The first part of this chapter portrays well the life and intellect of Wilhelm Conrad Röntgen. The second part contains personal reminiscences of Röntgen by Margaret Boveri, the daughter of Boveri the zoologist, who was Röntgen's colleague in Würzburg and probably his most intimate friend. These interesting reminiscences touch on Röntgen's youth and student life, his friends, the war, science, art and his last years. In this chapter are scattered many letters, some in facsimile, which give intimate contact, as it were, with a great personality. The reading of this most interesting chapter leaves the impression that it is a worthy account, sympathetic but truthful, of the life and character of Röntgen. Two quotations may be permitted by way of illustration:

"The salient feature of Röntgen's work . . . was his persistence and his critical honesty in making observations and measurements. He approached the solution of physical problems with great acuity and relentless thoroughness. Over and over again he worked out new control experiments in order to convince himself thoroughly of the absolute accuracy of the results which he had obtained, and with great scepticism he always warned against accepting any hypothesis which was not based upon sound experimental evidence. Therefore the results of his work which were published in his papers are distinguished by a rare reliability combined with remarkable classical brevity and simplicity." [Glasser, p. 74.]

"Perhaps Röntgen's most outstanding characteristic was his absolute integrity. Integrity in science, integrity in judging people even though appearances were deceiving, and integrity in faithful devotion to his friends though their opinions might be at variance. True, there were sometimes hardness and gruffness towards strangers and sometimes sudden anger toward those close to him, but these moments were overbalanced by the continuous warmth of his kindness and readiness to help, which ever again broke through the shy exterior and produced a deep and rare sense of security and confidence." [Margaret Boveri, p. 196.]

The remaining chapters are concerned with the various aspects of the early history of the roentgen rays and their applications. Chapter 23, the bibliography, gives a list of articles about Röntgen and lists of books, pamphlets and papers—1,044 separate items—on the roentgen rays, published in 1896. Glasser's book is a noteworthy addition to the history of science. The ninety-six illustrations add greatly to the value and interest of the presentation. In its special field it will be a great landmark and a source of information for all students of the roentgen rays and their discoverer.

**Die Spezifität der serologischen Reaktionen.** By K. Landsteiner. Price, 8.80 marks. Pp. 123, with 22 tables and 1 illustration. Berlin: Julius Springer, 1933.

Since 1875, when Landois first studied normal hemagglutinins, attempts to answer the question of the nature of antigens and antibodies have been so numerous that it is becoming more and more difficult for the student of immunology to separate the good from the valueless. To those who are trying with varying success to

keep up with the ever increasing avalanche of publications in this field, Landsteiner's monograph will be a welcome summary. Some long and particularly important chapters of books on modern immunology are the work of Landsteiner. No wonder that the book impresses one like a condensate of Landsteiner's life-work! His work serves as a background against which the important contributions of others are projected after they have been subjected to his critical evaluation.

The amount of information crowded into 95 pages of text shows marked ability to make every word count. This brevity and precision are laudable, and may be construed as an expression of his confidence in his readers. The value of the book is further enhanced by lucidity in the presentation of the material, none of which has been sacrificed for the sake of compactness. The reviewer does not recall having read an equally clear presentation of certain particularly complicated aspects of the chemistry of antigens.

The book is divided into six chapters. An analysis of the concept of serologic specificity is given in the introduction. The second chapter deals with the serologic specificity of proteins and with the effect of artificially produced chemical changes in the antigen. In the following chapter the specificity of cell antigens is presented, and the methods of differentiating between closely related species by means of cross-absorption are described. The iso-antibodies, the dissociation of bacteria, the heterophilic reactions, the lipoidal and carbohydrate haptens, the hypothetic structure of antigenic fractions of the cells and the serologic differentiations of individuals and species are also discussed. Then comes a chapter on the specificity of normal and immune antibodies. The degree of concentration in this chapter of 7 pages, with a bibliography of 97 references, may be illustrated by the fact that Ehrlich's hypothesis of the side chain is disposed of in a paragraph of 11 lines, and by the fact that only 30 lines are needed to give a clear presentation of the hypothesis of the origin of antibodies directly from the antigens. Chapter 5 is an admirable presentation of the serologic reactions with artificial complex antigens and simple chemical substances. While the book is not intended for the beginner, this particular chapter impresses the reviewer as a clear introduction to one of the most difficult branches of immunology. The last chapter summarizes the recent contributions to the present knowledge of specific substances of the cell, and is particularly concerned with the antigenic properties of carbohydrates and lipoids. The bibliography includes 966 references, each of which is considered in the text, and, in addition, a list of standard texts and monographs. The titles of 42 articles which appeared while the book was being printed are appended. The results of the author's recent and previously unpublished investigations are included, as, for example, a study of the distribution of the Forssman antigen (page 45) and an application of the azoprotein method to aliphatic compounds (page 83) and to peptides (page 85).

**Le poison des amanites mortelles.** By R. Dujarric de la Rivière. Price, 60 francs. Pp. 182, with 24 plates. Paris: Masson et Cie, 1933.

In the introduction the author discusses the history of poisonous fungi. Then follow chapters on the botanical description of *Amanita phalloides* and related forms; on the chemistry of the toxic principle; on the physiologic action of the poison; on the clinical phenomena and the structural changes following *Amanita* poisoning; on the medicolegal problems of poisonous fungi, including the detection of various fungi of this kind by study of the spores; on treatment and on prophylaxis, with particular reference to the conditions in France. There are twenty-four plates, four of which are in colors. At the end is a bibliography of some 500 references. The American literature receives adequate consideration. The monograph gives a thorough review of the present knowledge of poisoning by the "deadly *Amanita*."

## Books Received

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ANNUAL REPORT OF THE SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE OF THE UNITED STATES FOR THE FISCAL YEAR 1933. Price, 75 cents. Pp. 128. Washington: United States Government Printing Office, 1933.

ANNALS OF THE PICKETT-THOMSON RESEARCH LABORATORY. VOLUME IX. INFLUENZA (PART 1), WITH SPECIAL REFERENCE TO THE PART PLAYED BY PFEIFFER'S BACILLUS, STREPTOCOCCI, PNEUMOCOCCI, ETC., AND THE VIRUS THEORY. D. and R. Thomson. Price, \$8. Pp. 640, illustrated. Baltimore: Williams & Wilkins Company, 1933.

This is the first part of a huge monograph in two parts on influenza. The object is to summarize in convenient form the information in some four thousand publications on this disease. The present volume deals with the history, the clinical features and the epidemiology of influenza, but the greater part of it is devoted to the results of the investigations of the etiology. The literature on the relationship to influenza of Pfeiffer's bacillus, streptococci, pneumococci and other bacteria as well as of filter-passing "virus" is reviewed. The authors give an account of their own bacteriologic work on influenza which is illustrated with twenty-eight plates of photographs. The second part will deal "with the bacteriology of the complications of influenza as well as with the pathology, the treatment, and prevention of the disease," after which will follow "the vast bibliography of over 4000 references, as well as a very complete authors and subject index." Obviously the full scope and merits of the monograph will not become apparent until the second part is published.

LABORATORY MEDICINE: A GUIDE FOR STUDENTS AND PRACTITIONERS. Daniel Nicholson, M.D., Member of the Royal College of Physicians, London; Assistant Professor of Pathology, University of Manitoba; Assistant in Pathology, Winnipeg General Hospital, Winnipeg, Canada. Edition 2, thoroughly revised and much enlarged. Price, \$6.50. Pp. 566, with 124 engravings and 3 colored plates. Philadelphia: Lea & Febiger, 1934.

The first edition appeared in 1930 and was reviewed in the ARCHIVES (11:168, 1931). The second edition is an improvement on the first, and it presents well the indications, methods and interpretations of the diagnostic laboratory tests most suitable for current use in medical practice.

# ARCHIVES OF PATHOLOGY

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## CHEMOTROPISM OF LEUKOCYTES IN VITRO

ATTRACTION BY DRIED LEUKOCYTES, PARAFFIN, GLASS AND  
STAPHYLOCOCCUS ALBUS

MORTON McCUTCHEON, M.D.

WILLIAM B. WARTMAN, M.D.

AND

HAROLD M. DIXON, M.D.

PHILADELPHIA

It is generally accepted that the accumulation of leukocytes in areas of inflammation is due to chemotropism (chemotaxis). These terms mean that the cell orients itself in a concentration gradient of the attracting substance and advances (supposing the reaction to be positive) toward regions of higher concentration.

Previous experiments on chemotropism of leukocytes fall into two groups. Experiments of the earlier group were designed to ascertain whether more cells collected about substances tested for chemotropic properties than about indifferent substances. Usually a number of capillary tubes containing different substances were inserted in the tissues of animals or in suspensions of leukocytes. If, at the end of the experiment, a tube was found to contain more leukocytes than the control tubes, the substance in that tube was considered to be positively chemotropic; if, on the contrary, there were fewer leukocytes than in the control tube, the substance was regarded as *negatively chemotropic*.<sup>1</sup>

These older experiments have been so fully reviewed by Wells<sup>2</sup> that they need not be discussed here in detail. They yielded much information, not all of which is reliable. Thus it has been shown by Pfoehl<sup>3</sup> and by Ruchlädew<sup>4</sup> that under certain conditions leukocytes may be carried passively into the tubes by convection currents, in which case erroneous conclusions might be drawn as to the chemotropic properties

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1. For a description of other methods, the reader is referred to the review by Philipsborn (*Folia haemat.* 43:142, 1930).

2. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

3. Pfoehl, J.: *Centralbl. f. Bakt.* 24:343, 1898.

4. Ruchlädew, N.: *Ztschr. f. Biol.* 54:533, 1910.



of a given substance. (A further critique of this method is to be found in an article by Marchand.<sup>5</sup>)

In the second and more recent group of experiments a different criterion was used, namely, whether cells moved toward or away from the substance to be tested or whether they moved indifferently. These observations were made directly with the microscope.

Thus Commandon observed and recorded cinematographically the attraction of leukocytes by certain parasites in the blood<sup>6</sup> and by grains of starch.<sup>7</sup> Fenn<sup>8</sup> observed that leukocytes were attracted by particles of manganese dioxide but not by quartz.

Jochims<sup>9</sup> studied the reactions of leukocytes to solutions diffusing through a collodion membrane and found them to react positively to certain solutions and negatively to others. Schade and Mayr<sup>10</sup> made serial photographic records of cells moving toward dried drops of pus and drops of paraffin containing turpentine. With the same method, Schulz observed that leukocytes were attracted by certain bacteria and their products,<sup>11</sup> and Rosegger<sup>12</sup> reported the attraction in vitro of horse eosinophils by bits of parasitic worms.

Such direct observations on the direction of locomotion possess a great advantage over the older capillary tube method in that passive transportation of leukocytes (by convection currents) can be excluded; it may actually be seen whether the cell is moving by its own ameboid motion. However, it seems to us that such observations would gain in dependability and significance if the factor of random locomotion were controlled. The fact that a cell moves toward an object does not inevitably mean that the cell is attracted by it. We have observed leukocytes travel as far as 250 microns in a fairly straight line even in the absence of any known source of attraction.

Therefore, in the experiments reported here we sought to control the factor of random locomotion. This was done by counting the number of polymorphonuclear leukocytes that in unit time made contact with the attracting body; this figure was compared with the number of cells that made contact with an imaginary body of the same size and shape located in another part of the preparation.

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5. Marchand, F., in Krehl, L., and Marchand, F.: *Handbuch der allgemeinen Pathologie*, Leipzig, S. Hirzel, 1924, vol. 4, pt. 1, p. 336.

6. Commandon, J.: *Compt. rend. Soc. de biol.* **80**:314, 1917.

7. Commandon, J.: *Compt. rend. Soc. de biol.* **82**:1171, 1919.

8. Fenn, W. O.: *J. Gen. Physiol.* **5**:311, 1923.

9. Jochims, J.: *Arch. f. d. ges. Physiol.* **216**:611, 1927.

10. Schade, H., and Mayr, K.: *Krankheitsforschung* **8**:354, 1930.

11. Schulz, E.: *Ztschr. f. d. ges. exper. Med.* **84**:609, 1932.

12. Rosegger, H.: *Ztschr. f. d. ges. exper. Med.* **85**:712, 1932.

By this method we sought to answer the questions of whether cells are attracted by certain bodies or only accidentally make contact with them, and whether the accumulation of leukocytes about certain bodies is due to chemotropism or to the trapping<sup>13</sup> of cells which have been moving at random.

#### EXPERIMENTS

In the first series of experiments the attracting body consisted of a mass of dead (dried) leukocytes. This material was selected because in earlier (unpublished) work we had repeatedly observed that leukocytes frequently move toward, and make contact with, dead or immobile leukocytes accidentally present in the preparation. It seemed likely, therefore, that a clump of immobile leukocytes would strongly attract moving ones.

The attractive power of dead cells was tested by a modification of a simple method previously used for measuring the rate of locomotion of leukocytes.<sup>14</sup> Human blood was centrifugated, and from the buffy coat a minute drop of leukocytes was transferred to an ordinary glass slide. It was allowed to dry for several hours, and a flat body was obtained, about 400 microns in diameter. A drop of fresh blood from the same person was placed on a coverslip, lowered on the slide and allowed to spread in such a way that the clump of dried cells lay in the middle of the preparation. After the preparation was sealed with petrolatum, it was placed in the warm box at 37 C. We waited half an hour to allow the cells to develop normal locomotion. The clump of dried leukocytes was brought into the microscopic field and placed at the periphery. The image of the field was projected onto a piece of paper by a drawing ocular, as previously described,<sup>14</sup> and the position of each leukocyte was recorded at intervals of approximately one minute. In half an hour a record was obtained such as is shown in figure 1. Of the 9 cells that came into the field, 5 made contact with the mass of dried leukocytes, 3 moved out of the field and the remaining cell moved at random in the field. (Part of the path of this cell [2] has been omitted to avoid confusion.) It will further be observed that of the cells that made contact with the dried leukocytes, 1, 6 and 7 did so only after previous random movement. The impression gained from this experiment is that the leukocytes were attracted by the dried cells, but only weakly and from no great distance.

As a control experiment we determined the number of chance contacts made by cells wandering at random. For this purpose we drew on a fresh sheet of paper an imaginary attracting body of the same size and shape as the real one, and a field picked at random was projected

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13. A foreign body acts as a trap; cells coming in contact with it remain in the interface between the foreign body and the blood for a shorter or a longer time.

14. McCutcheon, M.: *Am. J. Physiol.* **69**:279, 1924.

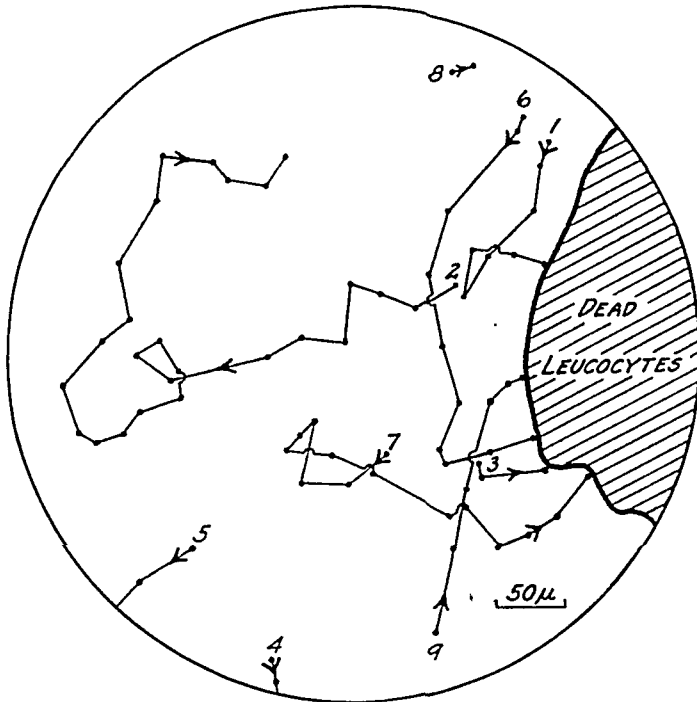


Fig. 1.—Camera lucida record of the paths of the 9 leukocytes that were seen in half an hour in a field containing a mass of dried leukocytes (shaded area). The dots represent the position of the leukocytes at intervals of approximately one minute. It is seen that during this time 5 of the cells (1, 3, 6, 7 and 9) made contact with the attracting body while the remaining 4 did not.

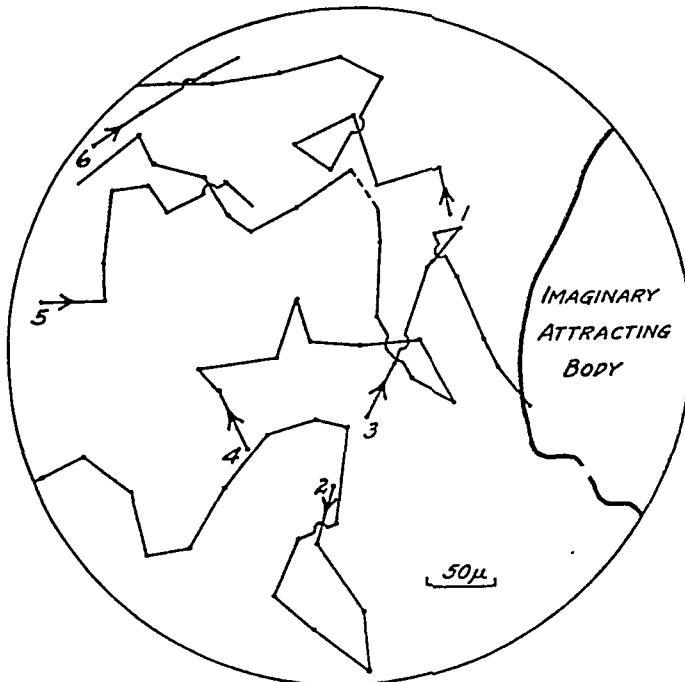


Fig. 2.—The attracting body is imaginary. Only 1 cell (3) made contact with it in half an hour; the locomotion of the cells was at random.

on it. The paths of leukocytes were recorded for half an hour, as shown in figure 2. It is seen that the paths are at random. (In several instances the complete path is not shown, in order to avoid confusion.)

Since it was found that cells coming into contact with the mass of dried leukocytes were usually trapped by it, cells "making contact" with the imaginary attracting body were treated as if trapped by it, and their further path disregarded. Thus, no cells were counted as making more than one contact with either the real or the imaginary attracting body. Any leukocytes that happened to be within the imaginary body were not counted. This procedure has the same effect as putting in the control field an actual object that exerted no chemotropic effect but that could trap leukocytes.<sup>15</sup>

In figure 2 it is seen that during the half hour 1 cell "came in contact" with the imaginary attracting body, as contrasted with 5 cells which made contact with the real attracting body. The procedure was then repeated both with the real and with the imaginary attracting body, so that at the end of the experiment the number of cells that had made contact with each in one hour was known. In the present experiment these numbers were respectively 6 and 1.

It is, however, not possible to draw any significant conclusion from one such experiment, since the number of cells is small and the difference in number of contacts might be obtained by chance. Therefore, the experiment was repeated a number of times.

In table 1 are summarized the results of ten such experiments. It is seen that in each experiment more cells made contact with the real than with the imaginary attracting body. The respective numbers for the entire series are 100 and 20, that is, five times as many cells made contact with the dried leukocytes as with the imaginary body in the control. In all, 202 cells were observed in the fields containing the real attracting body, of which 100, or 50 per cent, made contact. In the control fields, 170 cells were observed, of which 20, or 12 per cent, made contact.<sup>16</sup>

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15. The validity of this method depends on the fact that the field in the control experiment is chosen at random and a sufficiently large number of experiments made.

16. If correction were made for the difference in the number of cells in those fields containing dried leukocytes and in the control fields, the ratio of contacts in the two cases would be about 4:1 instead of 5:1 as given. But in later experiments, counts of the number of cells in different areas, made immediately after the preparation was placed in the warm box and before any migration occurred, showed the same number of cells near the attracting body as in distant fields. It therefore seems likely that in the present experiments the reason for the unequal distribution of cells was that more cells wandered from adjacent regions into the area containing the dried leukocytes than into the area containing the imaginary attracting body. In this case, therefore, no correction in the ratio is necessary.

We conclude from these results that leukocytes were definitely attracted by dried cells.

But before any significance can be attached to this result, the question must be answered as to whether the attraction was due to the dried cells in particular or whether any other solid material would have attracted the leukocytes as well.

Accordingly, in another series of experiments, instead of dried leukocytes we used such relatively inert and insoluble material as a

TABLE 1.—*Results of First Series of Experiments \**

Experiment	Number of Cells Making Contact with	
	Mass of Dried Leukocytes	Imaginary Body
1.....	6	1
2.....	9	2
3.....	11	4
4.....	18	1
5.....	4	0
6.....	13	3
7.....	5	2
8.....	11	2
9.....	13	2
10.....	10	3
Total hits.....	100	20
Cells making contact per hour.....	10	2

\* In each experiment the number of cells was counted that made contact with a mass of dried leukocytes in one hour (in experiment 3, seventy minutes), and compared with the number making contact with an imaginary body of the same size and shape in the same period of time. On the average, five times as many cells made contact with the dried leukocytes as with the imaginary body.

minute drop of paraffin wax, a bit of glass coverslip or a fine glass thread. Records were made showing the number of cells making contact with such an inert body and with an imaginary body of the same size.

In table 2 are summarized the results of twelve such experiments. Since not enough experiments have been made with different bodies to distinguish between them, they have been grouped together. It is seen that an average number of 6.6 cells per hour made contact with the actual body and 2.4 cells per hour with the imaginary body. This difference indicates that there is still an attractive force, though not as great as was exerted by the dried leukocytes.

Thus we have the result that comparatively insoluble substances sometimes definitely attract leukocytes. Similar observations have been made by others. Marchand<sup>5</sup> reported that leukocytes are attracted by fibrin; Philipsborn<sup>1</sup> saw them attracted by threads. Metchnikoff<sup>17</sup> found that amebocytes and leukocytes of various invertebrates collect around supposedly inert foreign bodies. Shaeffer<sup>18</sup> reported that amebas

17. Metchnikoff, E.: *Comparative Pathology of Inflammation*, London, Kegan Paul, Trench, Trübner & Co., 1893.

18. Shaeffer, A. A.: *Biol. Bull.* 31:303, 1916

TABLE 2.—Results of Second Series of Experiments\*

	Number of Cells Making Contact with	
	Paraffin	Imaginary Body
	4	0
	1	3
	1	2
	10	1
	12	4
	4	3
	10	2
	Glass	
	0	2
	11	0
	17	9
	2	1
	Fiber	
	7	2
Total hits.....	79	29
Cells making contact per hour	6.6	2.4

\* In each of twelve experiments, the number of cells making contact with a foreign body in one hour is compared with the number making contact with an imaginary body of the same size and shape in the same period of time. Combining the experiments, the ratio is about 2.7:1.

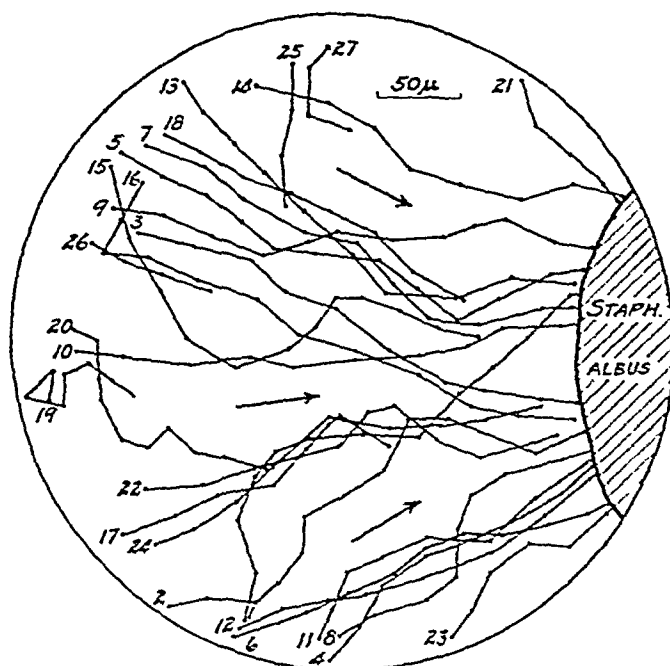


Fig. 3.—When the attracting body (shaded area) was composed of staphylococci, all the leukocytes advanced in nearly a straight line toward it. In half an hour 17 cells made contact.

thrust out pseudopods toward particles of glass and carbon from 60 to 100 microns distant.

The mechanism of such attraction is not known. In our experiments the following possibilities are suggested: 1. In some of the experiments the bodies may have been contaminated with a chemotropic substance. 2. Leukocytes making chance contact with the bodies were trapped and may have given off chemotropic substances. 3. The observation by Schulz,<sup>11</sup> that charcoal may take up a chemotropic substance from the medium and then attract cells, suggests the possibility of a similar mechanism in our experiments. 4. The bodies may have been sufficiently soluble to be themselves chemotropic.

The attractive force exerted by all the substances studied—glass and paraffin as well as dried leukocytes—seems to be quite definite, though certainly weak. For comparison with a strong chemotropic source, we present figure 3, in which the attracting body was a clump of *Staphylococcus albus*. In this figure it is seen that many leukocytes have entered the field and are all proceeding in more or less straight lines toward the attracting body. This is a typical example of the strong attraction which we found, in every experiment, to be exerted by *Staphylococcus albus* and a variety of other micro-organisms.<sup>19</sup> In seven experiments with *Staphylococcus albus*, the average number of cells making contact per hour was 22. By comparison with these micro-organisms, the attracting powers of dried leukocytes, paraffin and glass are seen to be weak.

#### SUMMARY

A method is described for studying the chemotropic response of leukocytes in vitro. The number of cells making contact with the attracting body in an hour is compared with the number making contact with an imaginary body of the same size placed at random in the field of leukocytes.

Dried leukocytes were found to attract polymorphonuclears weakly; about five times as many cells moved to the dried leukocytes as moved to an imaginary body of the same size.

Relatively inert substances like paraffin wax and bits of glass were also found by these methods to have a slight attraction for leukocytes. Possible mechanisms for such attraction are discussed.

Different kinds of micro-organisms were tested and all were found to attract leukocytes strongly. When *Staphylococcus albus* was used, all polymorphonuclears in the neighborhood moved to the bacteria in practically a straight line.

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19. The results of experiments with micro-organisms will be reported later.

# EFFECTS OF OXYACIDS AND HYDROXYACIDS ON PROTEIN SWELLING

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AND

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The notion that the action of an acid on the swelling of a protein colloid is the product of the hydrogen ions yielded by that acid when dissolved in water has been a dead issue to many workers in biology since early days,<sup>1</sup> yet its continuance into the literature of today by the followers of such workers as S. P. L. Sørensen,<sup>2</sup> Leonor Michaelis,<sup>3</sup> Henry R. Procter<sup>4</sup> and Jacques Loeb<sup>5</sup> justifies any renewed attack on the problem. The experiments detailed here on the effects of different acids on the swelling and "solution" of a highly purified globulin (a commercial Harris casein with 0.59 per cent of ash) may again be cited as quantitative evidence of the failure to find any relation between such effects on protein and the "strength" of the acids employed. They were not made, however, with this purpose first in mind but rather to get at an understanding of what might be the difference in the physiologic action of any simple acid as this was changed to the corresponding hydroxyacid or oxyacid. Thus the normal butyric acid of the fats consumed by man has a pathologic sister in the hydroxybutyric acid that appears in diabetes; propionic acid (from fat or protein), in the lactic acid discoverable in nonoxygenated muscle or in the body fluids in disease of the heart. In the plant world, on the other hand, acetic acid has a chemical teammate in the hydroxyacetic acid of unripe fruits, and succinic acid, in hydroxysuccinic (malic) and dihydroxysuccinic (tartaric) acid.

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From the Eichberg Laboratory of Physiology, the University of Cincinnati.

1. Fischer, Martin H.: *Am. J. Physiol.* **20**:332, 1907; *Œdema*, New York, John Wiley & Sons, 1910, pp. 31 and 33.

2. Sørensen, S. P. L.: Letter to the editor, *Kolloid Ztschr.* **40**:163, 1926.

3. Michaelis, L., in Alexander, J.: *Colloid Chemistry*, New York, Chemical Catalog Company, Inc., 1926, vol. 1, p. 498.

4. Procter, Henry R.: *Kolloidchem. Beihefte* **2**:270, 1911.

5. Loeb, Jacques: *Contributions to Medical and Biological Research*, Dedicated to Osler, New York, Paul B. Hoeber, Inc., 1919, vol. 2, p. 861. Bogue, R. H.: *Theory and Application of Colloidal Behavior*, New York, McGraw-Hill Book Company, Inc., 1924, vol. 1, p. 23.



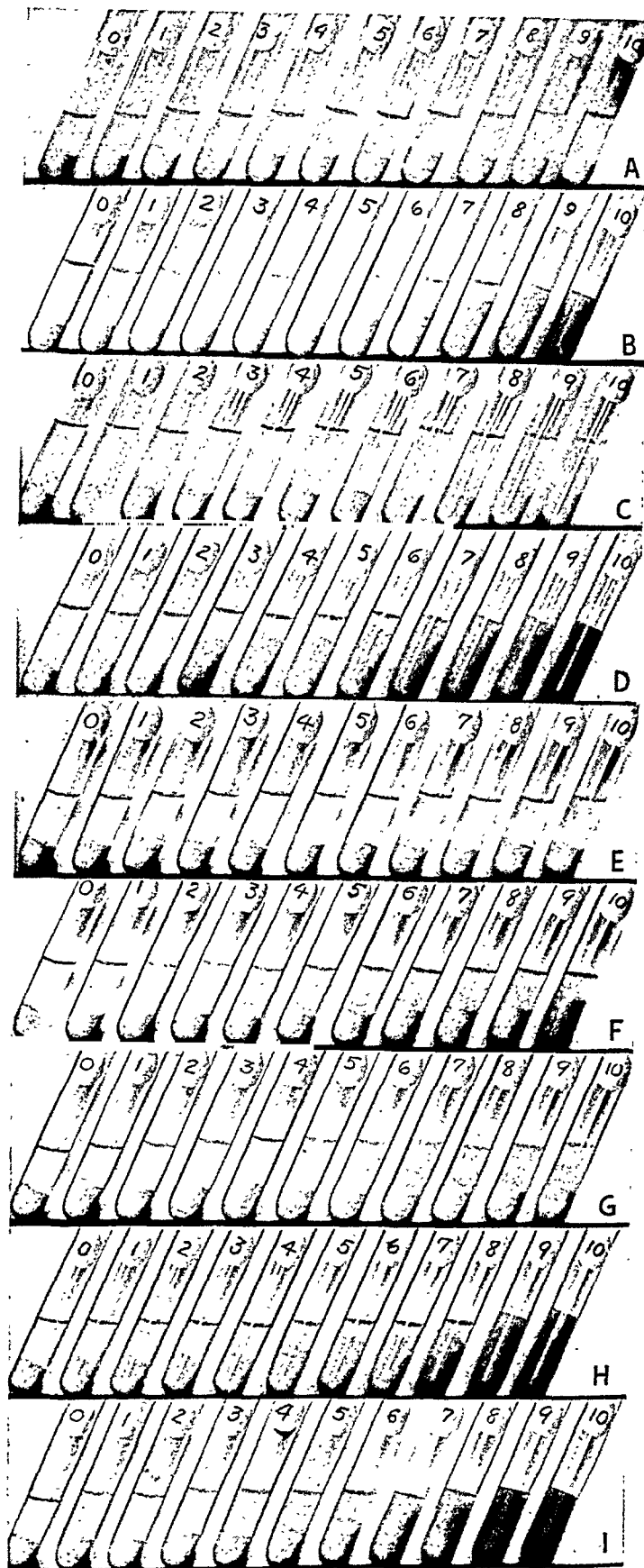


Fig. 1.—The several series of tubes show the effects of equimolar concentrations of different acids and hydroxyacids on casein. The successive series illustrate the effects of acetic, hydroxyacetic (glycolic), propionic, hydroxypropionic (lactic), butyric, hydroxybutyric, succinic, hydroxysuccinic (malic) and dihydroxysuccinic (tartaric) acids.

## EXPERIMENTAL DATA

Experiments showed that the hydroxylation of an acid generally increases the activity of that acid in producing the swelling and the "solution" of a protein (casein). Figure 1 *A* to *I* illustrates the effect better than many words. The tubes carry in all cases a unit weight of casein (0.625 Gm.) in a unit volume of "solvent" (5 cc.). The tubes marked 0 are controls, containing casein and water only. The others, numbered from 1 to 10, show the effects of increasing concentra-

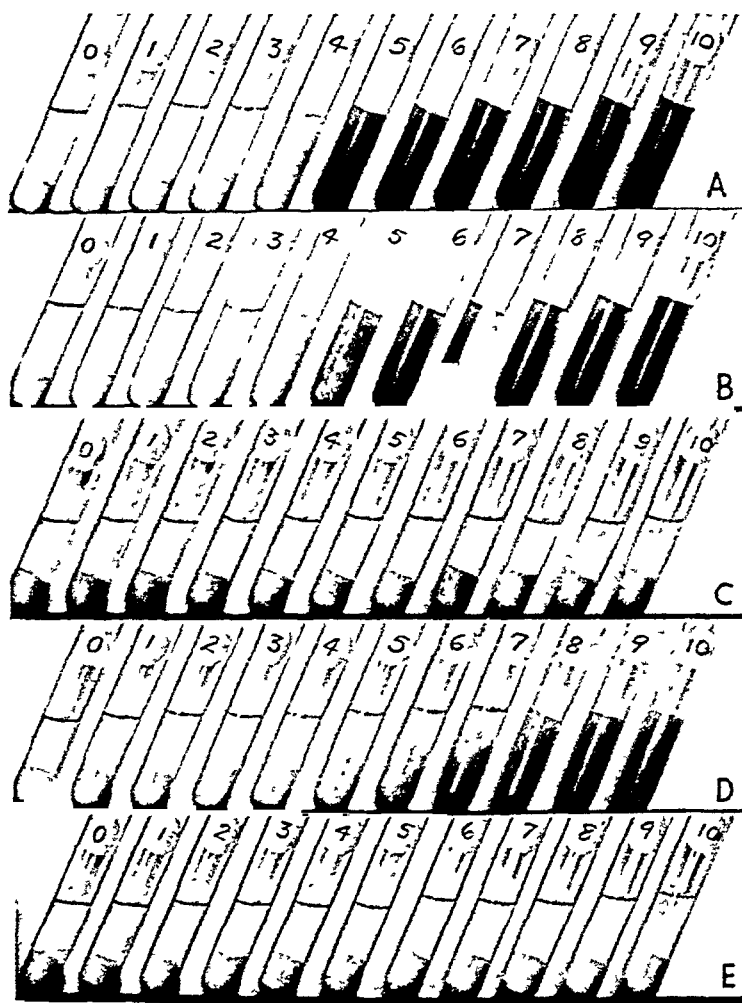


Fig. 2.—Effects of equimolar concentrations of several oxyacids on casein. The successive series illustrate the effects of phosphorous and phosphoric acids, and of hydrosulphurous, sulphurous and sulphuric acids.

tions of acid (in decimal increments from hundredth molar to ten-hundredths molar). Within this range of concentration acetic acid is seen to bring about almost no swelling (fig. 1 *A*). Hydroxyacetic acid, on the other hand, is so effective that increase in the height of the casein column is noticeable in tube 5 (fig. 1 *B*), while from tube 9 on the contents have swollen into solid gels. The effects of hydroxypropionic (lactic) as opposed to propionic acid may be seen by comparing figure 1 *D* with figure 1 *C*: that of hydroxybutyric against butyric acid,

by comparing figure 1 *F* with figure 1 *E*. In the series on succinic acid the barely appreciable swelling produced by the mother substance (fig. 1 *G*) becomes marked in hydroxysuccinic (malic) acid (fig. 1 *H*) and is further increased in the dihydroxyacid (tartaric) acid, as indicated by the shift of the swollen and gelled columns of casein toward the left in figure 1 *I*.

The effects of some oxyacids as opposed to the pure acids are illustrated in figure 2 *A* to *E*. Phosphorous acid (fig. 2 *A*) proves quite as active as phosphoric acid (fig. 2 *B*), both relatively and absolutely. Figure 2 *C* to *E* compares a series of the acids of sulphur. Hydrogen sulphide (fig. 2 *C*) is practically without effect in the concentrations here detailed. Sulphurous acid (fig. 2 *D*) produces a large effect, but this recedes practically to the level of no effect when sulphuric acid (fig. 2 *E*) is used.

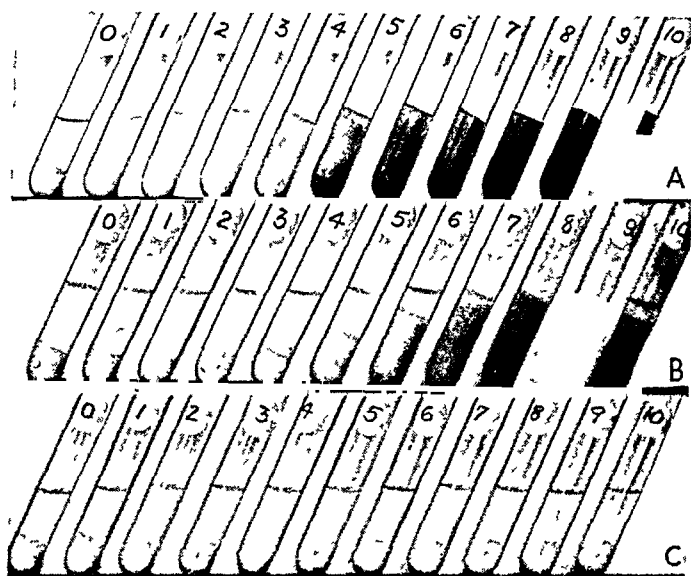


Fig. 3.—Successive series showing the effects of equimolar concentrations of hydrochloric, chloric and perchloric acids.

Figure 3 *A* to *C* compares the action of hydrochloric acid with two of the oxyacids of chlorine. Figure 3 *A* shows hydrochloric acid (of about the same  $p_H$  as the ineffective sulphuric acid of figure 2 *E*) to bring about an appreciable swelling in tube 3, after which its activity is so great that the contents of the remaining tubes set in solid gels. Chloric acid (fig. 3 *B*) though much "weaker" is not far behind, while perchloric acid (fig. 3 *C*) leaves the casein practically unaffected so far as its swelling and solution are concerned in the concentrations here employed.

#### CONCLUSIONS

Quantitative studies are detailed showing the effects of different acids on the swelling and "solution" of a highly purified casein. The action of the acids in no fashion parallels their electrolytic dissociation but each acts specifically. On comparing certain acids with their hydroxyacids it was found that the latter were uniformly more active. The effects of the oxyacids as contrasted with their mother acids cannot

be so easily compared. Little difference exists between the action of phosphorous and phosphoric acids; the acids of sulphur show greatest activity in a midzone of oxidation; of the acids of chlorine, hydrochloric is most active, with the oxyacids growing progressively weaker in this regard as the oxygen element is increased.

# AMYLOIDOSIS OF THE BONE MARROW

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The bone marrow is not a frequent site of amyloid deposits. When present in the bone marrow, amyloid is usually found in three forms, namely, isolated infiltrations of the vessels of the marrow in generalized amyloidosis, massive accumulations in the form of tumors, and deposits within preexisting blastomas or otherwise diseased bones.

In generalized amyloidosis the bone marrow is generally spared. Ponfick,<sup>1</sup> who first thoroughly investigated the bones for changes in amyloid disease, found but few instances of infiltration of the small and medium-sized arteries in the marrow. Eberth,<sup>2</sup> Wichmann<sup>3</sup> and others had the same experience. Askanazy,<sup>4</sup> who also methodically examined the bone marrow in cases of generalized amyloidosis, likewise reported only occasional instances of vascular infiltration.

A second form in which amyloid is found in the marrow is that of massive accumulations in the skeleton. These have been designated amyloid tumors of the bone, to indicate the tumor-like character of the amyloid deposit; they are not to be considered true blastomas of the bones. In these instances the amyloid deposits are more or less sharply localized in one or several sites in the skeleton. The uninvolved portion of the affected bone, as well as the remaining bones, is not infiltrated. The amyloid tumors of the bone have been described as occurring either alone or combined with amyloid deposits in the internal organs.

The third form in which amyloid is found in the bone marrow is that of a deposition of the amyloid within blastomas or otherwise diseased bones. In such instances the amyloid deposits are considered secondary to the underlying blastomas or chronic diseases, in contradistinction to the amyloid tumors of the bone which are considered primary or idiopathic. While the amyloid may be limited to the diseased bone, it is not infrequently found in the internal organs as well. The unaffected portion of the diseased bones and the remaining bones of the body are not infiltrated. The three forms of amyloid deposits in the bone marrow will be discussed later.

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From the laboratories of the Mount Sinai Hospital.

1. Ponfick, E.: *Virchows Arch. f. path. Anat.* **56**:534, 1872.

2. Eberth, C. J.: *Virchows Arch. f. path. Anat.* **80**:138, 1880.

3. Wichmann, G.: *Beitr. z. path. Anat. u. z. allg. Path.* **13**:487, 1893.

4. Askanazy, M., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1927, p. 835.

A survey of the literature has failed to disclose a description of diffuse amyloid infiltration of the bone marrow, that is, a diffuse involvement of the marrow comparable to that seen in the spleen or other hematopoietic organs. The following case represents such a condition.

#### REPORT OF A CASE

*History.*—A white man, aged 44, was admitted to the medical service of Dr. George Baehr at the Mount Sinai Hospital on March 29, 1928, complaining of pruritus, postprandial pyrosis, vague muscular pains and weakness. An examination revealed hepatomegaly, albuminuria, hypercholesteremia and a rapid disappearance of congo red injected into the blood stream. A diagnosis of amyloidosis, amyloid nephrosis, hypercholesteremia and lipid histiocytosis was made. Frequent observation showed persistent albuminuria and hypercholesteremia, the cholesterol rising as high as 0.625 Gm. per hundred cubic centimeters of blood. Roentgen ray examination of the bones revealed no changes. About two and one-half years after the first admission signs of renal insufficiency and hypertension supervened: these were attributed to incipient renal contraction secondary to amyloid nephrosis. At this time further roentgen studies revealed a collapse of the bodies of the ninth thoracic and first lumbar vertebrae. Biopsy showed a hyperplastic lymph node with lipid in some of the cells; the lipid was not doubly refractile and did not stain with sudan. Because of the changes in the lymph node and the hypercholesteremia a diagnosis of lipid infiltration of the bones was made. Hypertension persisted to the end. The renal insufficiency and vertebral lesions progressed until the patient died on March 10, 1931, of azotemia and pulmonary edema.

*Autopsy.*—An autopsy was done on March 10, 1931, four hours after death, by Dr. S. Otani and Dr. A. Schiffrin.

The body was somewhat emaciated; no jaundice, edema, petechiae or scars were noted. The lungs were edematous. The heart showed a moderate hypertrophy of the left ventricle. The liver was enlarged to about two and one-half times its normal size and weighed 3.720 Gm. Section revealed a dull, waxy, reddish-brown surface with indistinct lobular structure. (The reddish tint was ascribed to the retention of congo red administered during life.) The spleen was firm and weighed 140 Gm. The cut surface was pinkish red: the malpighian bodies were not distinctly visible. The pancreas and suprarenal glands appeared to be unaltered. The right kidney weighed 120 Gm. and the left 110 Gm. The surfaces appeared smooth: however, on examination with a hand lens fine granulation was detected. The cut surface was dull and waxy; the corticomedullary relationship was normal. The glomeruli were seen distinctly as small, bright red dots. Pinkish striations were seen coursing from the cortex to the medulla. (The glomeruli and tubules stained intravitaly with congo red.) The thyroid was firm and fleshy. There were healed erosions of the stomach and duodenum. Nowhere in the body were the lymph nodes enlarged. They were, in general, of the size of a pea, soft and on section characteristically grayish red.

*Vertebrae:* The anterior half of the bodies of the vertebrae from the eighth thoracic to the second lumbar was removed (fig. 1). The vertebral bodies were easily cut with a dull knife, except for the denser cortical portions just beneath the intervertebral disks. Instead of the network of spongy bone and red marrow usually seen, the cut surface was homogeneous, mahogany-colored, dull and waxy, and the framework of the spongy bone was not discernible. The marrow was of

putty-like consistency and, together with friable particles of spongy bone, could be readily scooped up on the back of a knife. Along the margins, especially the lateral, the cortical bone was less firm than normal and friable. The bodies of the ninth thoracic and first lumbar vertebrae were collapsed and deformed, resembling the vertebrae of a fish, and were only about one-fourth their original size. The margins of the collapsed vertebrae bulged laterally, while the surfaces adjoining the intervertebral disks, particularly the central portions beneath the expanded spongiosa of the disks, were depressed. Spicules of bone projected from the



Fig. 1.—Section of the anterior half of the bodies of the eighth, ninth, tenth, eleventh and twelfth thoracic and first and second lumbar vertebrae. Note the collapsed ninth thoracic and first lumbar vertebrae, with the expansion of the adjoining intervertebral disks. The marrow appears homogeneous; the framework of the spongy bone cannot be discerned.

depressed cortex into the pultaceous marrow. Above and below the collapsed vertebrae the intervertebral disks were elliptically expanded. There was no evidence of invasion of the surrounding muscle and connective tissue by tumor or amyloid.

**Other Bones:** The femoral marrow was reddish yellow, fatty and diffuent. The ilia possessed a dense, firm cortex, and the marrow was mahogany-colored, homogeneous and pultaceous; the spongy framework of the bone was not discernible. The ribs fractured readily, and the marrow resembled that of the vertebrae and ilia.

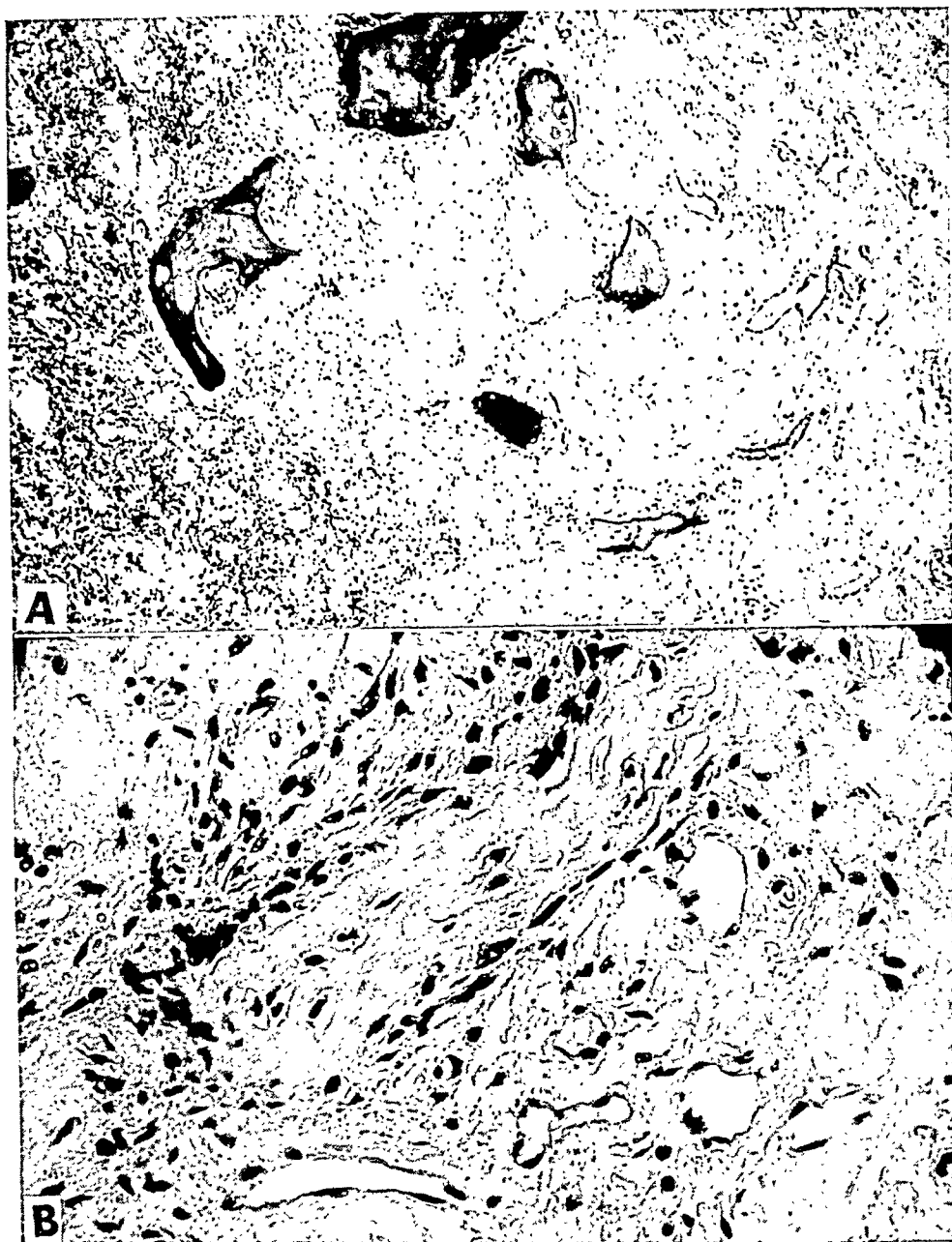


Fig. 2.—*A*, low power magnification of a vertebra, showing a central mass of amyloid and remnants of bony trabeculae. In the upper left corner the amyloid masses are seen infiltrated between the marrow cells, dividing them into islands. Note the regularity of the margin of the trabecula along which remnants of hematopoietic cells are still present. Hematoxylin and eosin. *B*, higher power magnification of a vertebra showing dilated sinusoids and fragment of a bony trabecula surrounded by amyloid. Large osteoclasts are seen along the margins of the trabecula; a multinucleated giant cell is present in a lacunar indentation. A small area of osteoid tissue can be seen just above the trabecula. Hematoxylin and eosin.



Because of permission for limited autopsy only, the brain and skull were not examined.

*Microscopic Examination.*—Vertebrae: Many sections were taken from the thoracic and lumbar vertebrae. Instead of the broad anastomosing lamellae there was a marked irregularity of the bony trabeculae, in both size and shape, and a replacement of the marrow by a mass of cell-free tissue. The cell-free tissue consisted of a structureless, homogeneous mass, generally taking a faint pink stain with eosin. Focal areas, in the vicinity of blood vessels, stained more deeply pink. The cell-free tissue was identified as amyloid by its staining reactions, particularly with the aniline dyes. Certain peculiarities of the amyloid staining reactions will be discussed in detail later.

The most marked infiltration of the bone marrow by amyloid, together with the most severe alteration of the spongy bone, was seen in the central areas of the vertebrae (fig. 2A). At those sites the amyloid was often arranged in more or less irregular islands demarcated by remnants of marrow cells. In the areas devoid of marrow cells the amyloid lay directly against the bony trabeculae. Numerous thin-walled, widely dilated sinusoids were present throughout. The amyloid in the walls of the sinusoids stained more intensely than the surrounding amyloid. The lumens of the arteries, small and large, were patent. The media of the arteries was homogeneous in appearance and stained positively for amyloid. Small, coarse clumps of amyloid were present in the adventitia and periadventitial tissues. The cells between the islands of amyloid were chiefly surviving hematopoietic cells, although occasional reticular cells and macrophages were seen.

Toward the cortex the surviving hematopoietic tissue was more abundant. Nevertheless, even at those sites the amyloid was seen to have infiltrated between the cells and divided them into irregular islands (fig. 2A). Directly beneath the cortex the marrow cells showed, as a rule, less amyloid infiltration; they were in normal relation to the bony trabeculae. The marrow cells showed a normal ratio of immature and mature myeloid and erythropoietic elements. There was no apparent change in the number of megakaryocytes; the reticular cells were discernible between the marrow cells. Aside from the occasional macrophages noted in the central areas of the vertebrae, no other adventitious cellular elements were noted.

The alteration of the bony trabeculae was most marked in the center of the vertebrae, where only small fragments of the spongy bone were seen. The margins of these remnants of bone were irregular and showed many lacunar indentations; the endosteum was disrupted. Closely applied to the irregular edges of the bone there was usually a row of large cells with abundant cytoplasm and a large nucleus that stained deeply blue with hematoxylin. In addition many multinucleated giant cells with tongue-like processes were seen along the margins of the bone and in the lacunar indentations (fig. 2B). Besides lacunar absorption many of the bony trabeculae showed advanced decalcification and resorption. There were scattered areas of osteoid tissue surrounding small fragments of bone (fig. 2B). Beneath the cortex the trabeculae of bone were generally large, broad and of regular outline; often, however, several showed early lacunar absorption or decalcification. The cortical bone was unaltered.

The destruction of the bony trabeculae was more or less sharply limited to those segments that were intimately surrounded by amyloid. No such alteration was noted where the bone was surrounded by marrow cells. In none of the sections were there evidences of the formation of new bone. There was no evidence of inflammation or tumor, in the bone, bone marrow or surrounding fibrous tissue.

The amyloid in the vertebrae did not stain with iodine or iodine and sulphuric acid. The amyloid in the media of the large and medium-sized arteries stained deeply red with congo red; the main mass of amyloid did not stain with this dye. With the aniline dyes a varied picture was observed. All the aniline dyes employed stained the amyloid equally well; cresyl violet, however, gave the most distinct picture. While the major portion of the amyloid in the center of the vertebrae stained a faint metachromatic pink, the scattered coarse clumps seen in the vicinity of the vessels and the amyloid in the media of the arterioles and walls of the sinusoids stained deeply violet. Between these two intensities there were many gradations in the metachromasia. Toward the periphery of the vertebrae and in

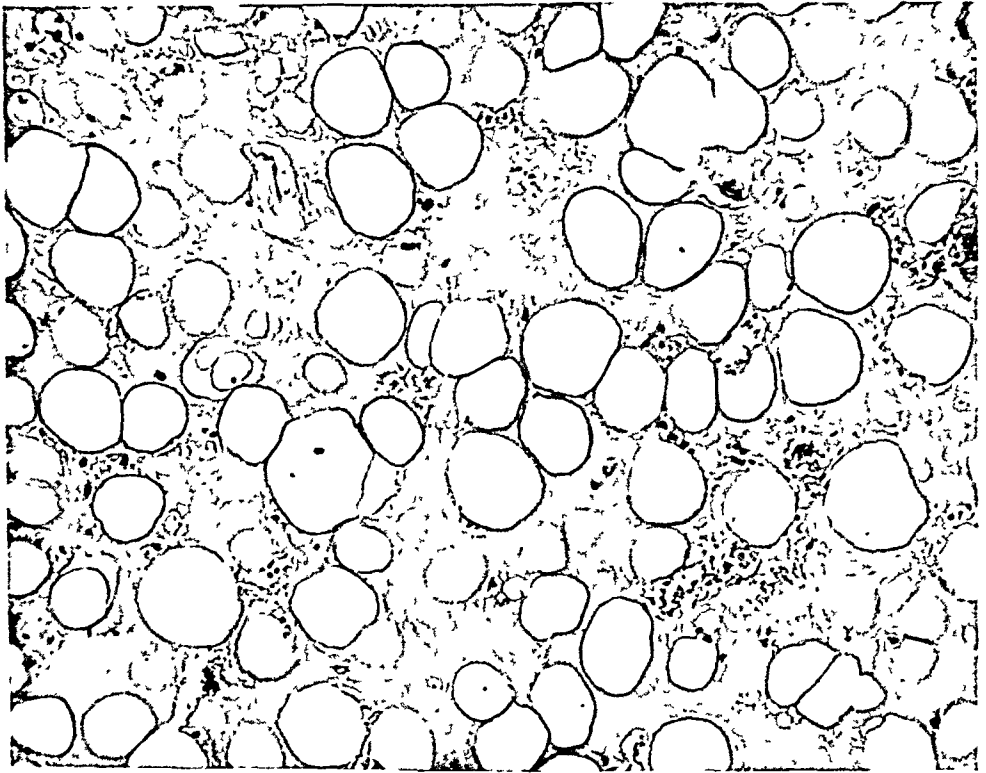


Fig. 3.—Fatty marrow of the femur showing diffuse amyloid infiltration between fat cells. Low power magnification. Hematoxylin and eosin.

between the large islands of hematopoietic tissue the amyloid stained more intensely, approaching a metachromatic purple. No amyloid was seen in the giant cells in the lacunae of the bone or in the macrophages.

**Marrow of the Femur:** There was a diffuse infiltration of amyloid in the spaces between the fat cells (fig. 3), and although a varying degree of compression of the fat cells was evident, no atrophy was noted. The hematopoietic elements showed the normal ratio of mature and immature cells. The distribution of the amyloid in the vessels, the alteration of the bony trabeculae and the staining of the amyloid were identical with the observations on the vertebrae.

**Kidneys:** The glomeruli were increased in size and markedly infiltrated with amyloid. The glomerular capillaries were seen with difficulty; they contained little blood. There was a marked increase of interstitial tissue, within which many

atrophic and collapsed tubules were seen. The remaining tubules were dilated; the epithelium showed marked granular degeneration, and the basement membrane was thickened by an infiltration of amyloid. The media of the small and medium-sized vessels showed marked amyloid deposition; the larger vessels were free. The lumens of all the vessels were patent. Some of the interlobular arteries showed a moderate degree of intimal proliferation and reduplication of the elastica; although carefully looked for, this change was not often observed.

**Lymph Nodes:** The architecture of the nodes was unaltered. The cortical follicles and lymphatic cords were depleted of cells. The sinuses were dilated and contained many macrophages and small lymphocytes. There was no evidence of lipid-containing cells. Amyloid was not present.

**Pancreas:** The interlobular and intralobular connective tissue was increased and diffusely infiltrated with amyloid, which involved also the media of the small vessels. The basement membrane of the acinar cells and the islets of Langerhans were not infiltrated. The large vessels and ducts were free from amyloid.

**Other Organs:** The liver, spleen, thyroid, suprarenal glands and the mucosa of the stomach showed the characteristic picture of diffuse amyloid infiltration with involvement of the small and medium-sized vessels. The other organs exhibited no significant change.

In none of the tissue sections was it possible to stain the amyloid with iodine or iodine and sulphuric acid. Except for the variations noted in the bone marrow, the amyloid in all other organs stained readily with congo red and the aniline dyes: cresyl violet, methyl violet and aniline gentian violet.

*Anatomic Diagnosis.*—The following diagnosis was made: amyloidosis of the liver, spleen, pancreas, stomach, thyroid and suprarenal glands; diffuse amyloidosis of the bone marrow with collapse of the ninth thoracic and first lumbar vertebrae; amyloidosis of the kidneys with incipient contraction; edema of the lungs; brown atrophy of the heart; hypertrophy of the left ventricle; healed erosions of the stomach and duodenum.

#### COMMENT

The diagnosis made during the life of the patient was amyloid nephrosis, hypercholesteremia, hepatomegaly and lipid histiocytosis. The clinical aspects of the case were reported by Rachmilewitz<sup>5</sup> while the patient was still alive. The subsequent postmortem observations of generalized amyloidosis, with marked involvement of the kidneys and liver, serve to explain the hypercholesteremia and hepatomegaly. No evidence of lipid histiocytosis was found. Despite a careful search at autopsy no explanation could be offered for the cause of the amyloidosis.

The amyloid deposits in the internal organs, aside from those in the pancreas and kidneys, showed nothing of unusual significance. The infiltration of the pancreas is noteworthy because of the infrequency of this condition. The microscopic appearance conformed to that seen in similar reported cases.

The clinical course of the patient presented a complete picture of amyloid disease of the kidneys beginning with the nephrotic syndrome and terminating with renal insufficiency. The signs and symptoms of

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5. Rachmilewitz, M.: J. A. M. A. 93:604, 1929

nephrosis persisted for a period of more than two years, during which the renal function and the blood pressure were normal. The gradual onset of renal insufficiency and the sudden appearance of hypertension were ascribed to incipient contraction of the amyloid kidneys; this was confirmed by the anatomic observations.

It has been observed frequently that in patients with amyloid contracted kidneys azotemia may develop terminally (Fahr,<sup>6</sup> Noble and Major<sup>7</sup> and Bell<sup>8</sup>). The occurrence of hypertension, on the other hand, has not been noted by the majority of observers (Volhard and Fahr<sup>9</sup> and Rosenberg<sup>10</sup>); they have reported a low tension as a rule. However, Fahr<sup>6</sup> recently asserted that hypertension may be present in young patients if cachexia is absent. He ascribes the infrequency of hypertension in patients with amyloid contracted kidneys to vascular "inadequacy" caused by cachexia, which is often marked in these patients. In these instances the hypertension is thought to be of renal origin. Similarly Bell, Noble and Major and others have reported hypertension with amyloid contracted kidneys and they consider the hypertension to be of renal origin. The case reported in this study seems to support this concept.

As mentioned at the beginning of this report, only scanty vascular infiltrations have been found in the bone marrow in cases of generalized amyloidosis. The nature of the infiltration, in these instances, does not differ from that seen in vascular involvement of other organs. In the present case, however, the amyloid deposits were seen not only in the vessels but also in the reticulum and in the walls of the sinusoids. The infiltration was comparable to that reported in the amyloid tumors of the bone. The latter, however, have many distinguishing characteristics. In contrast to the diffuse amyloid infiltration in the present case, the amyloid is localized as a massive accumulation in one or several bones while the marrow of the other bones is free. There are, however, a number of features common to both the primary amyloid tumors of the bone and the present case.

Eight instances of amyloid tumors of the bone have been reported (Edens,<sup>11</sup> Mandl,<sup>12</sup> Hildebrand,<sup>13</sup> Hedrén,<sup>14</sup> Schmid,<sup>15</sup> Ophüls,<sup>16</sup>

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6. Fahr, T.: *Klin. Wchnschr.* **10**:1205, 1931.

7. Noble, J. F., and Major, S. G.: *Arch. Path.* **8**:762, 1929.

8. Bell, E. T.: *Am. J. Path.* **9**:185, 1933.

9. Volhard, F., and Fahr, T.: *Die Brightsche Krankheit*, Berlin, Julius Springer, 1914, p. 21.

10. Rosenberg, M.: *Deutsche med. Wchnschr.* **57**:99, 1931.

11. Edens: *Virchows Arch. f. path. Anat.* **184**:137, 1906.

12. Mandl, J.: *Virchows Arch. f. path. Anat.* **253**:639, 1924.

13. Hildebrand, O.: *Virchows Arch. f. path. Anat.* **140**:249, 1895.

14. Hedrén, G.: *Ztschr. f. klin. Med.* **63**:212, 1907.

15. Schmid, H., cited by Askanazy.<sup>1</sup>

16. Ophüls, W.: *J. Exper. Med.* **5**:111, 1900.

Wolpert<sup>17</sup> and von Bonsdorff<sup>18</sup>). Two of these, the case of Schmid and that of Hildebrand, were instances of amyloid deposits within blastomas of the bone and should not be classified with the cases of amyloid tumors of the bone. Aside from the present case and the slight vascular involvement occasionally reported in generalized amyloidosis, the amyloid tumors of the bone represent the only other form of idiopathic amyloid disease of the bone marrow. Because of the destructive action of the amyloid deposits these tumors of the bone may give rise to serious symptoms and are often mistaken for blastomas. In all instances the authors described a cellular reaction in the area of the amyloid deposits, together with the presence of granulation tissue and multinucleated giant cells; these were considered to be local reactions to the presence of the amyloid. The alteration of the bone was comparable to that described in the present case. In only one instance was there evidence of vascular occlusion (Mandl); in the other cases the pressure of the infiltrating amyloid was probably responsible for the destruction of bone. This seems to be indicated by the studies in the present case. The most marked destruction of the bony trabeculae was noted in the areas in which the amyloid was present in direct apposition to the bone (fig. 2A). Where the marrow cells were still intact along the margins of the bone, that is, where the amyloid had not infiltrated to the bone, there was little or no evidence of destruction of bone. This seems to indicate that the direct pressure of the amyloid gave rise to the alterations in the bone. Neither in this case nor in the amyloid tumors of the bone was there any evidence of the formation of new bone. No explanation has been offered to account for this observation. The amyloid staining reactions in the amyloid tumors of the bone showed variations similar to those described in the present case; the amyloid stained irregularly with iodine and presented marked variations in metachromasia with the aniline dyes.

Amyloid deposits within blastomas or in chronic diseases of the bone or bone marrow are to be distinguished from the amyloid tumors of the bone. In the former conditions the amyloid deposits are considered to be secondary to the involvement of the bone marrow. When a tumor-like appearance is present, in contradistinction to the amyloid tumors of the bone, it is caused by the blastoma rather than the amyloid deposits. The quantity of amyloid present in the blastoma may vary from minute amounts, visible only under the microscope, to large accumulations sufficient to obscure the underlying blastoma. Weber<sup>19</sup> has

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17. Wolpert, I.: *Virchows Arch. f. path. Anat. (supp.)* **227**:173, 1920.

18. von Bonsdorff, B.: *Finska läk.-sällsk. handl.* **75**:447, 1933; abstr., *J. A. M. A.* **101**:489, 1933.

19. Weber, H.: *Beitr. z. path. Anat. u. z. allg. Path.* **86**:1, 1931.

emphasized the necessity of a careful search for the presence of the underlying blastoma; in a case reported by him, the multiple myeloma was found with difficulty. In these instances the destruction of the bone is no doubt primarily due to the underlying blastoma rather than to the amyloid, although the latter may aggravate the process. The staining reactions of the amyloid in blastomas are atypical, showing variations similar to those observed in the amyloid tumors of the bone and in the present case. Klemperer<sup>20</sup> described a case of lymphoblastic myeloma in which there were scattered homogeneous masses that stained deeply pink with eosin and partly yellow and partly red with van Gieson stain, but did not take the usual amyloid stains. It is possible that this was an instance of achromatic amyloid.

The blastomas most frequently showing secondary amyloid deposits are the multiple myelomas. Cases have been described by Weber, Glaus,<sup>21</sup> Hueter<sup>22</sup> and others. The relatively frequent association of this type of blastoma with amyloid deposits, both in the blastoma and in the viscera, has received the attention of many observers, who see in this association a pathogenetic relationship. Magnus-Levy<sup>23</sup> considers the Bence-Jones protein, occurring frequently in multiple myelomas, as the matrix of the amyloid. To advance his concept further, he suggests that the primary amyloid tumors of the bone are instances of multiple myeloma with secondary amyloid deposits rather than idiopathic amyloid tumors, the amyloid having arisen from the Bence-Jones protein. He likewise suggests that the amyloid deposits may, in these instances, have obscured the underlying blastomas, as Weber noted, or that the authors overlooked the underlying tumor as, for example, in Ophüls' case in which the amyloid deposits were considered to have arisen in a gummatous osteitis of the bones and were described by Ophüls as areas of plasma-like cells in the amyloid masses. There is nothing in the descriptions of the other cases of primary amyloid tumors of the bone, however, that indicates the presence of an underlying blastoma. The aforementioned authors stated that after careful search no evidences of tumor were found.

The diffuse distribution of the amyloid in the marrow readily distinguishes the present case from the other forms of amyloidosis of the bone marrow. Contrary to the observations in amyloid tumors of the bone, no inflammatory reaction was present despite the extensive destruction of the bones and the marked atrophy of the marrow. The involvement of the marrow was undoubtedly responsible for the secondary anemia observed in the later stages of the patient's illness. The

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20. Klemperer, P.: *Beitr. z. path. Anat. u. z. allg. Path.* **67**:492, 1920.

21. Glaus, A.: *Virchows Arch. f. path. Anat.* **223**:301, 1917.

22. Hueter, C.: *Beitr. z. path. Anat. u. z. allg. Path.* **49**:101, 1910.

23. Magnus-Levy, A.: *Ztschr. f. klin. Med.* **116**:510, 1931.

collapse of the vertebrae is comparable to that described in the case of amyloid tumor of the bone of the third thoracic vertebra reported by Mandl, in which pressure on the spinal cord had led to compression myelitis. It is not unlikely that in this patient the pressure of the collapsed vertebrae on the nerves as they emerged from the intervertebral foramina was responsible for the muscular pains in the lower part of the abdomen.

A careful study of the present case indicates that the amyloid deposits were not secondary to an underlying blastoma. In the mass of amyloid, even where the destruction of the bone was most extensive, the surviving marrow cells showed a normal ratio of myeloid and erythropoietic cells; no tumor cells were noted. Also the diffuse involvement of the marrow speaks against the presence of an underlying blastoma. However, because of the case reported by Freund,<sup>24</sup> it was necessary to consider the controverted concept of diffuse myeloma with secondary amyloid deposits. The presence of normal hematopoietic tissue throughout the marrow militates against this interpretation. The present case is clearly an instance of idiopathic diffuse amyloidosis of the bone marrow and viscera.

The rarity of amyloid infiltrations in the bone marrow has not been explained. Magnus-Levy offers an interpretation in the fact that the circulation of the marrow is constant and that, unlike the spleen and liver, it is not an expansile organ, so that the "pressing in" of circulating amyloid into the tissue spaces of the bone marrow is not favored. However, if the bone is the seat of a destructive process, as in blastomas, stasis ensues, and the deposition of amyloid is facilitated. This interpretation can account for only some of the physical factors, as Magnus-Levy himself acknowledges. It cannot, however, be applied to instances in which destruction of the bone was not present prior to the amyloid deposition, as in the present case.

#### SUMMARY

In addition to the three described forms of amyloid deposits in the bone or bone marrow, namely, isolated instances of vascular infiltration in generalized amyloidosis, primary amyloid tumors of the bone and secondary amyloid deposits within blastomas or other diseases of the bone, a fourth type is added, that of diffuse amyloidosis of the bone marrow associated with generalized amyloidosis. No similar case was found in a survey of the literature. No cause for the amyloidosis was found.

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24. Freund, E.: *Frankfurt. Ztschr. f. Path.* **40**:400, 1930.

# A PRINCIPLE ACCELERATING GROWTH AND MATURATION

DEMONSTRATED IN METASTASES OF A TUMOR OF THE THYROID GLAND

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The physiologically active principle characteristic of the parental tissue has been demonstrated in a variety of tumors. Thus, epinephrine was demonstrated by the frog's eye method and chemically in medullary tumors of the suprarenal gland by Orth,<sup>1</sup> Wiesel and Neusser,<sup>2</sup> Wegelin,<sup>3</sup> Hedinger,<sup>4</sup> Raben<sup>5</sup> and Schultz<sup>6</sup> and by the effect on blood pressure by Hick.<sup>7</sup> Plaut<sup>8</sup> demonstrated the presence of thyroxine in thyroid gland rest tumors of the ovary by the acetone nitril (Reid Hunt) and the tadpole maturation (Gudernatsch) tests. Heller<sup>9</sup> and many others observed bile in the metastases of hepatocellular carcinomas of the liver. Similarly, certain clinical observations suggest that tumors may display the functional properties of the parental tissue. In this category fall the Langhans cell tumors with hyperparathyroidism,<sup>10</sup> the oxyphilic and basophilic adenomas of the hypophysis<sup>11</sup> and the gonadal tumors with their respective syndromes dependent on the elaboration of the principle characteristic of the tissue from which they originate. The demonstration of prolactin A in the urine in teratoma testis<sup>12</sup> and that of intermedin in the urine in neurogenic tumors and melanomas<sup>13</sup> are of even more direct significance. Similarly, Ewing<sup>14</sup> mentioned

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1. Orth, K.: *Preuss. Akad. Wissensch.* **2**:34, 1914.
2. Wiesel, J., and Neusser, E.: *Die Erkrankungen der Nebennieren*, Vienna, M. Perles, 1910, p. 27.
3. Wegelin: *Verhandl. d. deutsch. path. Gessellsch.* **15**:255, 1912.
4. Hedinger, E.: *Frankfurt. Ztschr. f. Path.* **7**:112, 1911.
5. Raben, C. B.: *Arch. Path.* **7**:228, 1929.
6. Schultz, W. H.: *Am. J. Physiol.* **90**:508, 1929.
7. Hick, F. K.: *Arch. Path.* **15**:665, 1933.
8. Plaut, A.: *Klin. Wchnschr.* **10**:1803, 1931.
9. Heller, cited by Ewing.<sup>14</sup>
10. Jaffé, H. L.: *Arch. Path.* **16**:63 and 232, 1934.
11. Cushing, H.: *Bull. Johns Hopkins Hosp.* **50**:137, 1932.
12. Ferguson, R. S.: *Am. J. Cancer* **18**:269, 1933.
13. Ferguson, R. S.: *Am. J. Cancer*, to be published.
14. Ewing: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1928, p. 53.



absence of symptoms of Addison's disease in a patient with a massive hypernephroma and apparently complete destruction of the suprarenal glands. In this instance the evidence is not so certain, since aberrant suprarenal tissue is common and symptoms do not develop in the presence of excessive amounts of cortical substance.

Ewing summarized this subject by stating that the functional activity displayed by tumors diminishes with increasing anaplasia. Any amount of function from overactivity to complete absence of activity may be met with.

The pathologic entity variously referred to as metastasizing thyroid gland, metastasizing goiter or metastasizing adenoma has been thoroughly studied from the morphologic standpoint and, indeed, the various names applied to it serve to indicate its malignant character as observed grossly and clinically, and its benign character as observed histologically. Engelstad<sup>15</sup> demonstrated active thyroid substance in the metastases of a carcinoma of the thyroid gland by the acetonitril test. In the present article I shall present evidence of a substance promoting growth and maturation which was present in the metastases of a tumor of the thyroid gland, the primary growth of which was histologically malignant, though at autopsy the thyroid tissue present and the metastases as well were histologically benign.

#### REPORT OF CASE

In 1920 a white woman, 49 years of age, noted a slight enlargement on the anterior surface of the neck, which increased to the size of a hen's egg during the following two years. During the third year the mass began to increase rapidly and the patient complained of a choking sensation when she attempted to swallow. During this period she lost about 10 pounds (4.5 Kg.), but did not complain of nervousness, intolerance of heat or other features characteristic of hyperthyroidism. She entered the Cook County Hospital on July 4, 1923, at which time the right lobe of the thyroid gland was enlarged to the size of a grapefruit. It was firmly fixed to the surrounding structures and moved only slightly during swallowing.

Lobectomy was performed and sections of the tumor were examined microscopically. The growth was diagnosed as carcinoma of the thyroid gland.

Roentgen examination of the chest, spine and long bones failed to demonstrate metastases.

The patient was readmitted in June, 1932 (nine years after the original admission and twelve years after the onset of symptoms). At this time she complained of sacro-iliac pain. An x-ray picture revealed metastases in the sacrum. Six months later (Jan. 19, 1933) she again returned to the hospital with a history of having had headaches and "pistol shot" noises in the head for eight years, a swelling in the right parietal region for four years, and weakness and dyspnea becoming progressively more pronounced for several months.

She was extremely emaciated. A soft, smooth elevated, pulsating mass, 12 cm. in diameter, was present in the right parietal bone. A ragged edge, apparently

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15. Engelstad, R. B.: *Ztschr. f. Krebsforsch.* **39**:369, 1933.

bone, was palpated about the margin of this mass. A loud bruit was heard over the dilated and tortuous temporal vessels, and similar but softer sounds were audible over the tumor itself. The vessels on the right side of the neck pulsated, and over them a thrill was felt and a bruit heard. The heart was enlarged and a double murmur was heard over the apex. The upper region of the right lung gave a dull sound on percussion, and the breath sounds were of diminished intensity and associated with sibilant râles. The basal areas posteriorly gave a dull sound and here many moist râles could be heard. The finger tips were clubbed and cyanotic.

The veins in both fundi were engorged, and papilledema was noted on the right.

The laboratory findings were insignificant.

A rapidly downward course terminated in death on January 25 (six days after admission).

*Autopsy.*—Dr. R. H. Jaffé made the following observations eight hours after the death of the patient. The external findings were essentially as described in the foregoing paragraphs.

The pleural cavities were partially obliterated by dense adhesions, and each contained about 100 cc. of clear fluid. The lungs were distended and heavy, and the pleural surfaces were covered with fibrous tags. The surfaces were studded with firm, light purple-gray nodules, varying in size from that of a pin-head to 20 mm., the larger of which displayed central umbilication. Similar masses were scattered throughout the lung and, as seen on the cut surface, were separated by moist, light purple-gray to purple-brown pulmonary tissue.

The pericardial sac contained 100 cc. of clear fluid. The heart weighed 415 Gm. The myocardium was pale and friable. Atheromatous and calcareous plaques were scattered over the inner surfaces of the coronary arteries and aorta.

The left lobe of the thyroid gland weighed 35 Gm. The color varied from light purple-gray to grayish brown. The cut surface was made up of nodes from 20 to 30 mm. in diameter and it varied in color from light brown to yellowish brown. To the right of the trachea in place of the right lobe of the thyroid gland was found a firm, confluent mass of spherical nodes 8 by 5 by 4.5 cm. in diameter. The sectioned surface of this mass was light purple-gray mottled with grayish white.

In the fundus of the stomach at the greater curvature was a firm submucous nodule 25 by 15 by 10 mm. in diameter.

The mass noted on the external aspect of the skull was found to extend through the bone and to be firmly adherent to the dura. The edges of the bony defect were indented and friable. A similar mass protruded into the pelvis from the lower half of the sacrum.

*Microscopic Examination.*—In the thyroid gland the follicles were generally small, lined by epithelium varying from flat to cuboid and largely isolated into nodules by a dense hyaline and, in some areas, calcified stroma. In certain areas the follicular epithelium contained blood pigment. The superior thyroid artery and its branches displayed marked calcification. The veins were distended, but no fragments of the tumor were found in the lumens.

In the lung the nodules seen grossly were sharply demarcated from the surrounding compressed and, in some areas, blood-filled alveoli. They consisted of a delicate vascular stroma and numerous small follicles resembling those seen in the nodules in the thyroid gland. The smaller follicles had practically no lumens; in the larger ones the lumens were empty or filled with pale-staining homogeneous

material. The cells lining the follicles were uniform in size, shape and staining qualities. Hemorrhage was seen in the centers of some of these metastatic nodules.

The peritracheal lymph nodes showed follicle-like structures separated by a varying amount of stroma replacing the normal lymphatic tissue. The follicles were lined by cylindric epithelium displaying a moderate degree of papillary infolding.

The metastases to the parietal bone presented essentially the same histologic picture as did the pulmonary metastases.

A nodule from the region of the gastric fundus was examined. The mucosa was thickened. It was made up of long, slender glands lined by regular, clear cylindric epithelium and other irregular, branched structures formed by large cells with hyperchromatic and anaplastic nuclei as well as dilated glandlike structures the lumens of which were filled with pus cells and lined by low cylindric epithelium. Single glands extended through to the serosa. The stroma was rich in small round cells and plasma cells.

*Anatomic Diagnosis.*—The anatomic diagnosis was: metastasizing goiter involving both lungs, the right peritracheal lymph nodes, the parietal bone and the sacrum; ancient lobectomy of the right lobe of the thyroid gland; early carcinoma in adenomyoma of the stomach; chronic tumor of the spleen; moderate hypertrophy of the heart; parenchymal degeneration of the liver, myocardium and kidneys; mucous colitis and bilateral hydrothorax.

#### EXPERIMENTS

Several days after the death of the patient the nodules of the tumor were dissected out of one lung. They totaled 250 Gm. in moist weight. They were run through a meat grinder, spread on glass plates and dried in a stream of warm air. On April 23, 60 mg. of this dried material was added to the fish food placed in each of two pans, one containing seventy and the other forty-five tadpoles. This daily ration of material from the tumor was continued until July 13. Several variable-sized groups of tadpoles from the same batch of eggs were retained as controls and fed only fish food or fish food plus dried beef heart. The experimental group and the controls were killed with chloroform on July 13, and the maximum length of each tadpole was measured and the animals were photographed (figs. 1, 2 and 3).

The mean and the standard deviations were calculated according to the formulas described by Pearl.<sup>16</sup>

In the control group of eighty-four tadpoles, the mean length was  $2.39 \pm 0.036$  cm., and the standard deviation was  $0.49 \pm 0.025$  cm. Group 1 of the tumor-fed tadpoles with sixty-four survivors at the end of the experiment had a mean length of  $3.64 \pm 0.1$  cm., with a standard deviation of  $1.19 \pm 0.071$  cm., and, in addition, six fully developed frogs and one tadpole with hindlegs at the termination of the experiment. Similarly, group 2 of the tumor-fed tadpoles with forty

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16. Pearl, R.: Introduction to Medical Biometry and Statistics, Philadelphia, W. B. Saunders Company, 1930, chap. 13, p. 264.



survivors at the end of the experiment had a mean length of  $5.19 \pm 0.119$  cm., with a standard deviation of  $1.112 \pm 0.084$  cm. Three fully mature frogs and four frogs with tails retained developed in this group.

Thus the control group showed a standard deviation of only 0.49 cm., indicating a uniform and rather narrow distribution of sizes about the mean of 2.39 cm., and no maturation during the experimental period. Group 1 of the tumor-fed group with a standard deviation of 1.19 cm. about the mean of 3.64 cm. and group 2 with a standard deviation of 1.112 cm. about the mean of 5.19 displayed a much more rapid and variable rate of growth. The time of maturation, as is indicated by the development of fully or partially matured frogs in these two groups, was also accelerated. The difference in growth and maturation between

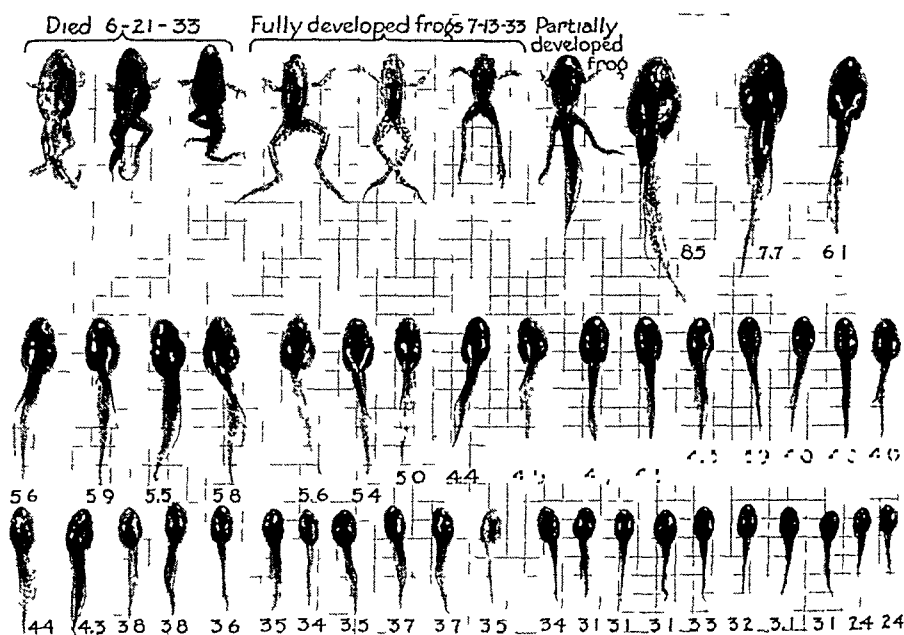


Fig. 3.—Group 2 of the tumor-fed tadpoles, given the same rations over the same period as group 1. Forty out of forty-five survived. The mean length of the survivors was  $5.19 \pm 1.19$  cm. The standard deviation was  $1.112 \pm 0.084$  cm.

the two tumor-fed groups is explainable by the difference in the number of tadpoles in the two groups, the smaller group showing the most rapid growth and maturation.

The variations within the group are explainable by differences in the individual intakes and differences in the developmental potentialities of the individual tadpoles. Which of these factors was the chief or perhaps the sole element could have been settled only by placing one or two tadpoles in each experimental pan instead of the large numbers used.

Because of the length of time intervening between the death of the patient and the preparation of the dried material of the tumor, quanti-

tative estimates of the potency of this substance would have been futile. However, 0.06 mg. of commercial desiccated whole thyroid gland given daily killed a pan of tadpoles within one week whereas the number killed by 0.06 Gm. of the tumor substance, while higher than the number dying among the controls, was only a small fraction of the total number of tadpoles given this dose.

These findings allow the conclusion that the metastases of the tumor of the thyroid gland contained a principle that accelerated the growth and maturation of the tadpoles.

#### SUMMARY

Experimental evidence is presented that there exists in the metastases of tumors of the thyroid gland a principle that accelerates the growth and maturation of tadpoles.

# THE INHERITANCE OF FOCAL MELANOSIS IN DROSOPHILA

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PRINCETON, N. J.

In a study of the physical characteristics of the gene,<sup>1</sup> adult male drosophilas were exposed to one minute irradiation with roentgen rays from a copper target tube. Eight thousand nine hundred and twenty electrostatic units were given per minute per square centimeter in 1 cm. of air by this tube. These males were bred to females having in one chromosome the familiar C1B factor combination. The female progeny containing as their sex chromosomes the C1B of the mother and the irradiated X of the father were bred in single cultures to males having sex chromosomes containing the genes for white eye color and miniature wings. The progeny of one such mating, 16546 A-20, were fifty wild type and fifty-four Bar females and two males. Both males showed black melanotic spots on the second joint of the legs. They walked stiffly and easily became stuck in the culture mediums. These males were bred to their wild sisters which carried the mutant gene. There resulting progeny included a very few females, as well as males, which had black spots on the second joint of the legs. The normal wild type male and female of *Drosophila* are shown in figure 1 *A* and *B*. Two females and a male showing the degenerated leg joints are shown in figure 1 *C*, *D* and *E*.

A sex-linked lethal gene kills off all the males which receive it, or one half of the total male progeny. In most instances no trace of these males is discovered. The existence of a lethal gene is inferred from the fact that a genetic class which should occur is missing. In the case described in this report a few of the males carrying this gene are not badly affected and survive. They represent only a small proportion of the expected males. In the initial case in which there were fifty wild type and fifty-four Bar females there should have been fifty males having lesions to correspond with the fifty wild type females, whereas only two survived. Examination of the culture bottle revealed the bodies of the rest of these males, each one showing pronounced lesions.

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From the Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J., and the Department of Biology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.

1. Gowen, John W., and Gay, E. H.: Gene Number, Kind and Size in *Drosophila*, *Genetics* 1:1, 1933.

The males having this sex-linked gene had been able to crawl out of the pupa case but, unable to move normally, had died. The paper at the bottom of the bottle covered with these dead males is shown in figure 1, *F*. Sixty-two males showing lesions of the joints were found dead on the paper. When they were all counted the males were of sufficient number to balance the female class, as they should be to meet genetic expectations.

The extent to which degeneration may go is shown in figure 1 by the size of the blackened area which replaced the leg joints. This degeneration apparently develops at the time the fly leaves the pupa case, since melanotic lesions were not noted in flies of the proper genetic constitu-

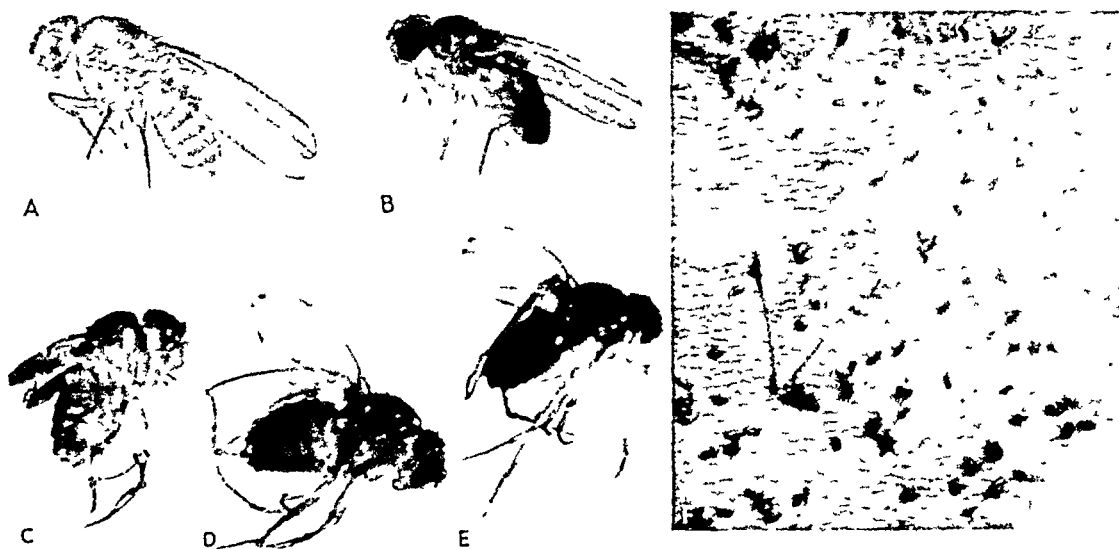


Fig. 1.—Normal wild type male and female for comparison with males and females showing lesions of the leg joints, an inherited pathologic condition produced by a gene. Roentgen treatment of the wild type gene of the wild type normal flies shown in *A* and *B* caused a change into a gene producing the condition shown in *C*, *D* and *E*. The males which show the effects of this gene generally die. In *F* their bodies are seen scattered over the paper which covered the bottom of the bottle in which they emerged from their pupa cases.

tion dissected from the pupa cases. The lesions do not show any increase in area with the aging of the fly. There is no evidence of continuous growth.

The amount of tissue included in the focal melanosis varies from culture bottle to culture bottle, environmental conditions such as moisture playing a rôle in the expression of this gene. In its host expression the blackened necrotic area may appear as only one slight spot on the lower tip of the femur or upper end of the tibia. In its more extreme



condition all the legs may be involved, the necrotic degeneration including half the femur and tibia on either side of the joint. No melanosis of any of the other joints, as those between the tarsae or coxa, trochanter and femur, takes place. Figure 2 shows three leg joints displaying these lesions; the joint in *2 A* has quite a limited melanosis, that in *2 B* is more extreme, and in *2 C* the melanosis involves about one third of both the femur and the tibia.

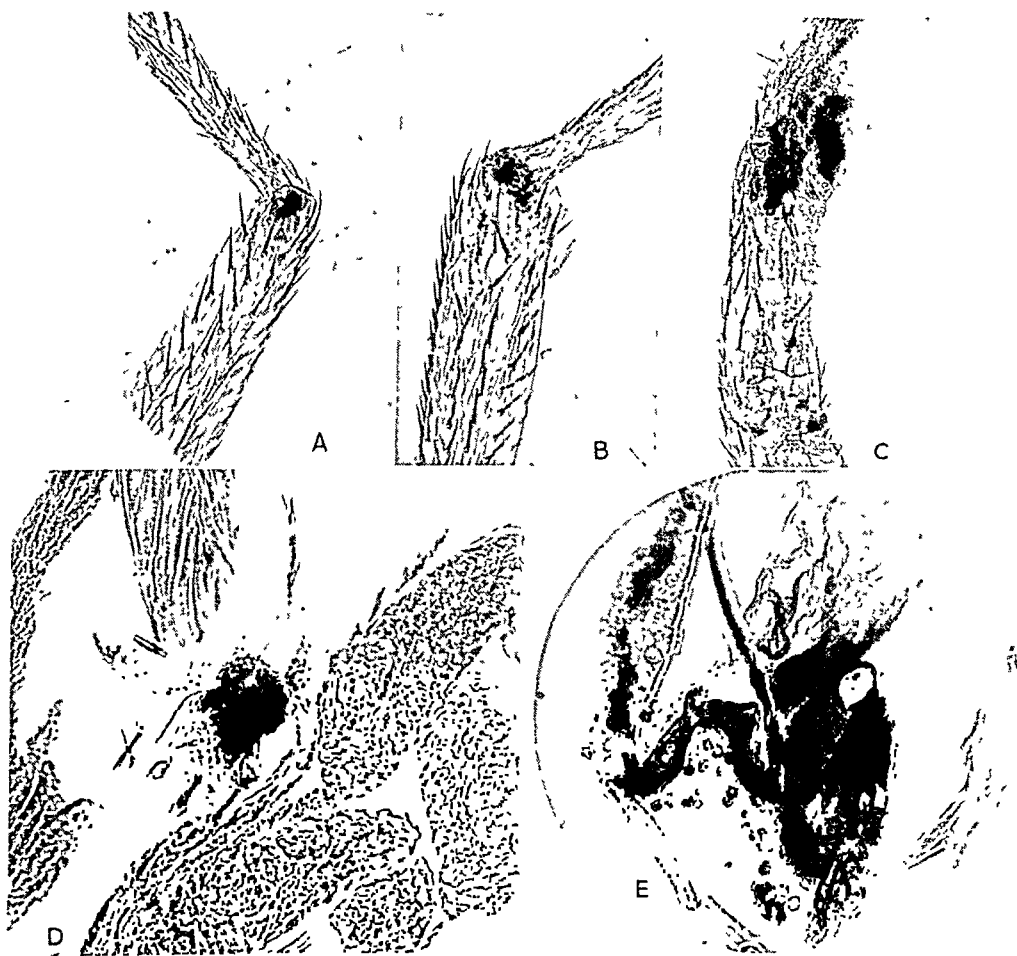


Fig. 2.—Various types observed in the focal melanosis of the leg joint. *A* shows but slight melanosis; *B* shows a more extreme, and *C* a still more complete, involvement of the leg. *D* and *E* are photographs of sections through these joints. *D* is a section stained with eosin. As the melanotic area is pigmented black with insoluble pigment it appears black in the photograph. *E* is a section at a higher magnification and is stained with iodine gentian violet, a nuclear stain. Again the melanotic area is black owing to the pigment.

High power magnification of sections through the necrotic areas shows that the region of functional failure and degeneration is at the junction of the tibia and femur. The degeneration includes the cuticula and the underlying ectoderm. The muscles do not appear to be involved

except in the lesions in which degeneration is extensive, when the whole femur and tibia may be reduced to a structureless mass of melanotic tissue. In such cases muscular atrophy appears to be a secondary change brought about by the antecedent cuticular degeneration.

The chemical solubilities<sup>2</sup> of the black pigment left after degeneration of the tissues are of interest as they give some idea of its nature. Soaking the specimens for a period of two years in water, weak hydrochloric acid, ethyl alcohol, ether, xylol, carbon bisulphide, glycerin, glacial acetic acid, purified petroleum benzine and solutions of calcium hydroxide and barium hydroxide failed to dissolve the pigment. It is readily soluble in a 5 per cent solution of sodium hydroxide, leaving a clear chitinous shell. It is also soluble, with some difficulty, in concentrated nitric acid, the chitinous shell dissolving as well as the black pigmentation. To this extent, then, the degeneration may be regarded as replacing much of the tissue with a substance having the general properties of melanin.

The technic used to detect mutations produced by roentgen rays showed the gene causing this focal melanotic degeneration to be sex-linked. Its further linkage relations are shown in the following tabulation.

Parents	♀	$\frac{y_1 w_2 m \cdot Bx}{me}$	♂	y	cv	v	f
		Progeny					
Genetic constitution							
noncrossovers				Without melanotic lesions		With melanotic lesions	
y w m Bx.....				157			
+ .....				7		27	
Single crossovers							
y							
w m Bx.....				3			
y w.....						1	
m Bx.....				4		1	
y w m.....				7			
Bx .....						1	
Double crossovers							
y m Bx							
w							
y Bx							
w m							
y w Bx							
m .....				2			

The data list the males only. Of the alternate classes for each crossing over, the classes showing the yellow gene are given first. Crossing over in the presence of this gene has obviously been seriously reduced.

2. Abel, J. J., and Davis, W. S.: On the Pigment of the Negro's Skin and Hair, *J. Exper. Med.* 1:361, 1896.

The evidence shows the gene to be between white and miniature, near miniature. Crosses including the factors  $y\ cv\ v\ f$  show a like result.

Parents	♀	$\frac{me}{y_1CV_2V_3f}$	x ♂ y cv v f	
			Progeny	
Single crossovers				
Genetic constitution		♂'s	♂'s	♀'s
noncrossovers		Without melanotic lesions	With melanotic lesions	Without melanotic lesions
y cv v f.....		258		197
+ .....		1	12	213
Single crossovers				
y .....				1
cv v f.....		1		1
y cv.....				1
v f.....		2		
y cv v.....		7		8
f .....			1	6
Double crossovers				
y v f				
cv				
y f				
cv v				
y cv f				
v				

The inherited factors other than the factor for the focal melanosis, *me*, are the familiar ones: yellow (*y*), white (*w*), miniature (*m*), Beadex (*Bx*), cross-veinless (*cv*), vermilion (*v*) and forked (*f*), distributed along the length of the sex chromosome.

The data in the foregoing tabulation agree with those of the previous cross in showing the gene's locus to be at the left of vermilion and near it. So far as the flies which may be counted as live adults are concerned, it acts nearly as a lethal factor. It is evident that the chromosome change also markedly affects the rate of crossing over, the amount being reduced from 56 per cent for the yellow to forked region to only 4 per cent. The crossing over rate in the yellow to vermilion region is normally 33 per cent. In the presence of the gene, tentatively designated as *me*, it is reduced to 0.7 per cent. The vermilion to forked region suffers a reduction to 3.3 per cent from a normal of 23 per cent. This reduction in crossing over is not primarily due to the gene for melanotic lesions as is shown from data on an independent mutation produced by roentgen rays in the same locus. This second mutation came as the result of a later experiment with roentgen rays. Males containing mature sperm were treated with 26,620 electrostatic units from a chromium target tube. These males were bred as in the first experiment. The  $F_2$  male progeny showed the same black pigmented spots as those of the first mutation. In all respects the somatic effects of this gene

resembled those of the first. Flies containing the second gene were crossed with those having the first gene. The progeny showed the genes to be the same or at least allelomorphic. The linkage test showed the following crossover values with yellow cross-veinless vermilion forked. The yellow to cross-veinless crossover ratio was 9.4 per cent; cross-veinless to *mc*, 17 per cent; *mc* to vermilion, 3.8 per cent, and vermilion to forked, 28 per cent. Considering the total amount of the data, sixty flies, all of these percentages are well within the standard values. The locus of the gene for the focal necrosis of the femur-tibia joint may consequently be placed in the sex chromosome at 29. This locus corresponds with that of another gene, described some years ago as producing a somewhat similar effect. This ascute gene was located at 28 units.

The mode of inheritance of focal melanosis of the leg joints has made it possible to maintain the disease for over one hundred generations of progeny since the first fly with lesions appeared. During this time no fly showed any trace of the lesion unless it carried the genetic constitution for it. As in every culture bottle about half the population was without the gene producing focal melanosis, this half acted as normal controls for the other half having it and showing it by the lesions of the leg joints; it follows that interpretation of the disease as contagious is ruled out.

That the melanosis is dependent on the proper genetic constitution of the given body cells is critically demonstrated by a fly representing an accident of development. The left side of this fly was male and had only one sex chromosome which chanced to carry the gene for this melanotic degeneration of the joints. The right side of the fly was female and had two sex chromosomes, one carrying the gene for the focal melanosis, the other having the yellow, white, miniature and Beadex factors as well as the normal allelomorph of the focal melanosis gene. In crosses the focal melanosis gene behaves as a recessive. One would therefore expect that since it is homozygous on the left, or male side, of the fly that focal melanosis would be noted on this side. Such lesions were found, the melanosis on the second and third legs being rather extreme. Similarly on the female side, where the cells are only heterozygous for the focal melanosis gene, one would expect no lesions. Careful examination of the femur-tibia joints showed them to be perfectly healthy. The fortunate loss of the yellow, white, miniature Beadex chromosome from half of this fly's body has thus shown clearly the dependence of focal melanosis on the genetic constitution of the particular cells in the region where degeneration is to take place.

#### COMMENT

Focal melanosis of the femur-tibia junction in these flies has been shown to have its ultimate etiology in a single sex-linked factor. This gene's locus within the chromosome is about 29 units from the left end.

The disease-producing factor appeared as a result of a specific alteration in gene material located within a minute region of the sex chromosome. The agent initiating these changes was roentgen rays from copper in one case and from chromium in another. The melanosis is completely suppressed in the heterozygous animal by the action of the normal allelomorphic gene within the nuclear structure of the same cell. In a fertilized egg this gene for focal melanosis is presumably carried to each cell of the developing embryo by the successive divisions of the cell. It reacts only at the time when the processes of differentiation are essentially completed as a specific degeneration of a given group of cells. Since such a gene is capable of being carried to any of the future generations of progeny by breeding, it follows that the focal melanosis has unlimited possibilities of spread in the population.

This statement of the conclusions to be drawn from the evidence for the germinal origin of the lesions seems clear. The manner in which the gene acts in the embryologic development of the organism is not so evident. From examination of the formed fly as it is found in the late stages of pupal development one finds no traces of the melanotic focal lesions grossly evident in the imago. There may be some slight thinning of the legs at the joints, but there are no obvious lesions. The condition appears to arise rather suddenly at or near the time of emergence of the fly from the pupa case. Flies which show the lesions when kept alive for a period of time show no growth of the involved area. From these facts the case for the embryologic changes involved may be regarded somewhat as follows: The normal potentialities for growth differentiation of the leg joints are present and are exercised in the embryologic development of the affected flies. These tissues when formed have a defective vitality owing to the presence of the gene under discussion. In the stress of emergence from the pupa and in favorable environmental conditions the defective tissue of this strictly localized area is changed to form the pigmented residue noted as the end-product of the reaction. Such changes, while they strike the imagination because they are disease-producing, are not new. Like changes of the end-products of gene control in embryologic development are familiar enough in normal development as, for instance, the differentiation of no pigment versus brown pigment in the eye of man. It is not commonly seen in cases like that described here, owing, I presume, to the fact that, should the lesions involve vital organs in the early development, little or no trace of the embryo would be left to study. As indicated elsewhere<sup>3</sup>

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3. Gowen and Gay.<sup>1</sup> Morgan, T. H.; Bridges, C. B., and Sturtevant, A. H.: *The Genetics of Drosophila*, The Hague, M. Nijhoff, 1925, p. 10. Muller, H. J.: *The Problem of Genic Modification*, Proc. 5th Internat. Genetics Cong., Ztschr. f. indukt. Abstammungs-u. Vererbungslehre (suppl. vol.) 1:234, 1928.

it is just such cases which are most frequently noted in the gene mutations studied in genetic research.

The study of intra-uterine amputations in man leads Streeter<sup>4</sup> to like views, although in those cases the evidence for the genetic nature of the defects cannot be made so precise owing to the technical difficulties of the material. The embryologic evidence leads to the view that these amputations are of germinal origin. In these cases sharply circumscribed areas of limb-bud tissue are of such inferior quality that only imperfect histogenesis occurs. These areas maintain themselves only in the earlier weeks of pregnancy. By the fourteenth week they are fibrous moribund masses, and when these slough away, the limbs show depressions, grooves or healed stumps.

Similar focal deficiencies have been noted in cattle. Hadley<sup>5</sup> has shown that the progeny of certain animals of the Johanna and Sarcastic Lad families of Holstein-Friesian cattle produce offspring with congenital epithelial deficiencies. These deficiencies tend to be localized in the extremities. Frequently the epithelium is not intact below the knees or hocks or on the muzzle, mucous membrane of the nostrils, tongue, hard palate and cheeks. The ears and dew claws are often deformed. Animals with this defective epithelium are unable to survive, since bacteria easily penetrate the open surfaces and produce fatal septicemia. The genetic data indicate that this type of focal deficiency is due to a single mendelian factor.

Focal deficiencies may include constituents of a circulating tissue as well as those of the fixed tissues. Such a deficiency occurring in mice was formerly thought to be completely lethal<sup>6</sup> but has since been shown by De Aberle<sup>7</sup> to be a focal deficiency of the hemoglobin of the blood. The mice having the genetic factor for this deficiency in a homozygous condition have only about a third the hemoglobin present in the blood of normal mice. The deficiency brought about by this gene may be obviated in part by the injection of normal blood into the mouse with a defective genetic constitution, as shown by Gowen and Gay.<sup>8</sup>

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4. Streeter, George L.: Focal Deficiencies in Fetal Tissues and Their Relation to Intra-Uterine Amputations, Washington, D. C., Carnegie Institution, 1930, publ. 414, p. 1.

5. Hadley, F. B.: Congenital Epithelial Defects of Calves, *J. Hered.* **18**:487, 1927.

6. Little, C. C.: The Inheritance of Black-Eyed White Spotting in Mice, *Am. Naturalist* **49**:727, 1915.

7. De Aberle, S. B.: Hereditary Anemia in Mice and Its Relation to Dominant Spotting, *Am. Naturalist* **59**:327, 1925.

8. Gowen, John W., and Gay, E. H.: Physiological Factors Necessary to Alleviate Genetic Lethal Anemia in Mice, *Am. Naturalist* **66**:289, 1932.

The cases which seemingly most nearly parallel that described here are those of tumors in larva and adult *Drosophila* as described by Stark.<sup>9</sup> Two different tumor-like growths have been described. The first of these is characterized as an irregular darkly pigmented growth of epithelial origin. The cells near the center are large, polyhedral, spheroidal or fusiform. Pigment is present both within and outside the cell. Toward the periphery the cells become flattened and closely crowded together, forming laminated layers of pigment giving to the tumor the appearance of encapsulation. The growths may occur in the proventricular ganglion, salivary glands or imaginal rudiments of the thorax and abdomen. Larvae carrying these growths never survive to pupate. The ultimate agent which causes the growths to appear is a gene, lethal 7, located at 0.3 in the sex chromosome. It is limited to flies which have the proper genetic constitution, no evidence for any secondary effect of an outside pathogen being manifest.

This case of lethal 7 differs from focal melanosis of the femur-tibia joint in having a much wider range of foci in which its effects may manifest themselves. The tumor condition is quite toxic; larvae showing the tumor seldom live more than fifteen hours, whereas flies with the focal melanosis of the leg joints live for quite a long period, providing the lesion is not so extreme as to prevent locomotion. The pigment tumors produced by the lethal 7 gene show a continuous increase in size while under observation. The lesions of the leg joints, on the other hand, appear to remain of the same size once they are produced.

The second growth studied by Stark<sup>9</sup> is a benign tumor dependent for its expression chiefly on two genes, one in the third chromosome and one in the second chromosome. These pigmented growths may occur in any segment of the larva. The cells are rounded or polygonal and pigmented. If, perchance, the tumor cells happen to invade the imaginal disks of the appendages, they check the development of these parts. A larva showing the growth may pupate, emerge as an adult, and still carry the tumor. The growth visibly increases in size.

In a number of their effects these growths resemble those of the focal melanosis of the leg joints as here described. There are, however, equally important differences which are, I believe, sufficiently marked to suggest a different mode of action for the genes producing the defects. The fact that the lesions of the femur-tibia joint appear after the fly emerges as an adult, when it is known that the joint is completely formed in the pupa, suggests a distinctly different process from that seen in the abnormal growths of lethal 7, etc. This view is strengthened by the fact that the lesion, although variable in extent, does not seem to

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9. Stark, M. B.: An Hereditary Tumor in the Fruit Fly, *Drosophila*, J. Cancer Research 3:279, 1918.

alter its size when flies having it are isolated and kept for two or three weeks. The fact that when examined in the pupa this joint appears thinner than normal—although apparently normal in its tissues—gives still further weight to this view. The chain of observations suggests that this gene causes the formation of cuticular tissue of inferior quality, although apparently normal in appearance, and that this tissue, in the strain and stress of emergence from the pupa case, alters its course of development, giving focal degeneration of the leg joints. The parallel seems to be greater between this lesion and those observed in man by Streeter<sup>1</sup> than between it and the tumors described by Stark.

#### SUMMARY

This report presents a study of a focal melanosis of the femur-tibia articulation in *Drosophila melanogaster*. The focal melanosis is induced through the action of a gene located in the middle portion of the sex chromosome, 29 units, mutated twice by roentgen rays, once by 8,920 electrostatic units from a copper target tube and again by 26,620 electrostatic units from a chromium target. The lesion is grossly evident in the imago shortly after emergence, as a dark melanotic degenerate mass at the articulation of the femur and tibia. The effect is somewhat variable. It may involve one or all the legs to a variable extent. Flies having the focal melanosis are usually found dead. The condition is in no way contagious to other flies. Sections show that the melanotic areas are localized at the ends of the femur and tibia in the cuticular layer and its underlying ectoderm. In chemical solubilities the black pigmented masses left in the tissue degeneration behave like melanin pigment.



# THE AGE OF PREGNANCY

## HISTOLOGIC DIAGNOSIS FROM PERCENTAGE OF ERYTHROBLASTS IN CHORIONIC CAPILLARIES

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AND

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A simple, reliable method of estimating the age of pregnancy from histologic examination of placental fragments would satisfy more than the mere academic interest of the pathologist. Its medical and legal benefits appear obvious. Various methods reported in the past have apparently missed application in routine laboratory work because of their dependence on qualitative criteria as standards of comparison, unvarying technic in the preparation of material and individual experience in judgment. Variations in the multiplicity and size of chorionic villi, differences in the density of their stroma and the number of outer layers were employed to distinguish between early and late pregnancies.

By means of the present study we wish to call attention to a histologic method of determining the age of pregnancy early in its development, which has proved its practical worth in our laboratory since its introduction by Dr. Kornel Terplan three years ago. Based on the maturation of red blood corpuscles in the fetus, with their development from nucleated to nonnucleated forms, and a differential enumeration of these elements in chorionic capillaries, this method palpably eliminates personal factors in interpretation and furnishes results which can be expressed in quantitative terms. The *Quarterly Cumulative Index Medicus* and the monographs of Meyer<sup>1</sup> and Frank<sup>2</sup> reveal no reference to a systematic investigation identical with ours. Williams,<sup>3</sup> however, in a discussion of placental chronology, wrote: "It should be remembered that during the first 8 to 10 weeks of pregnancy the blood cells of the fetus are nucleated, but afterward the nuclei are lost."

### PROCEDURE

One hundred consecutive specimens labeled "placental tissue" and bearing trustworthy clinical data were received following delivery, curettage, abortion or operations for tubal pregnancy. Each specimen was fixed for sixteen hours in 10 per

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1. Meyer, Robert, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, vol. 7, p. 649.

2. Frank, R. T.: *Gynecological and Obstetrical Pathology*, ed. 2, *Gynecological and Obstetrical Monographs*, vol. 12, New York, D. Appleton and Company, 1931, vol. 12.

3. Williams, J. W.: *Obstetrics*, New York, D. Appleton and Company, 1930.

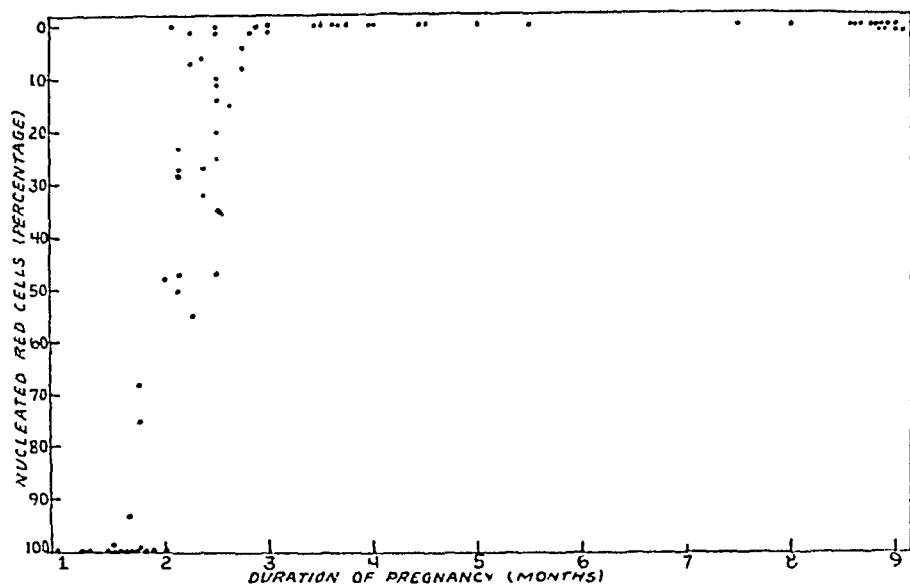


Fig. 1.—Percentage of nucleated red blood cells in the chorionic villi of seventy placentas of various ages.

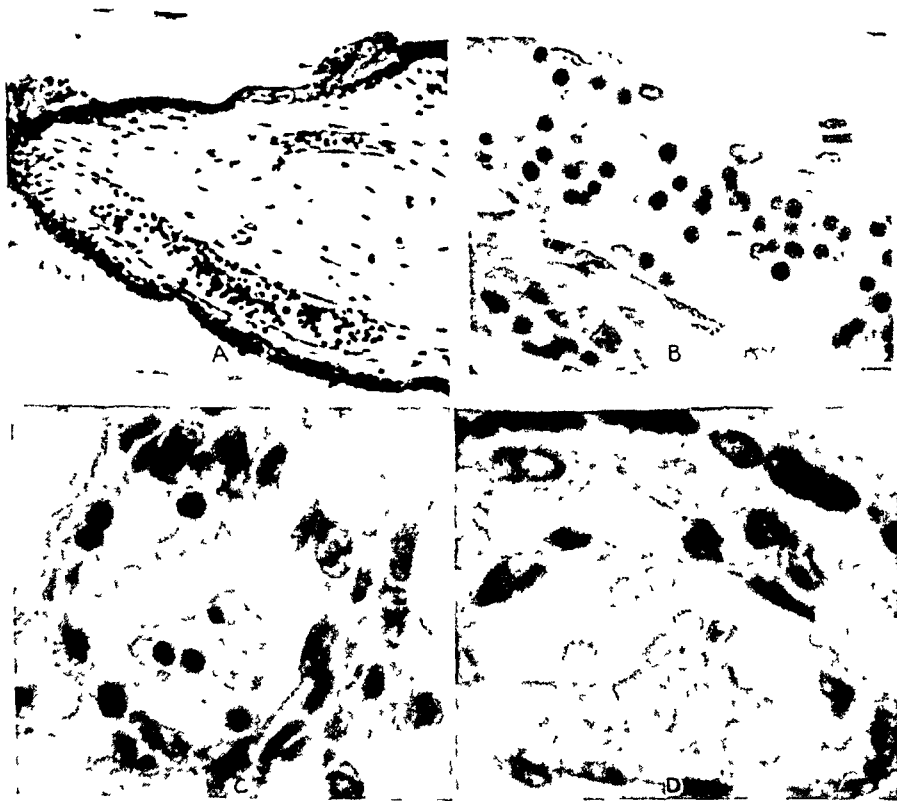


Fig. 2.—*A*, villus showing capillaries filled with nucleated red cells (low power magnification); *B*, corpuscles in a chorionic capillary in a six weeks' pregnancy (immersion in oil); *C*, corpuscles in a chorionic capillary in a two months' pregnancy (immersion in oil); *D*, corpuscles in a chorionic capillary in a pregnancy of three months or over (immersion in oil).

cent formaldehyde. After being dehydrated in alcohol and chloroform, the tissue was embedded in paraffin. Sections were cut 8 microns in thickness and stained with hematoxylin-eosin. With low power magnification, villi containing capillaries were located. Then from 100 to 200 red cells were counted while the specimens were immersed in oil, the whole process consuming not more than a few minutes for each slide. The proportion of nucleated and non-nucleated cells was calculated in percentages. In several cases differentiation of megaloblasts from normoblasts was attempted. The age of pregnancy was deduced from the patient's history, conception being postulated as having occurred seven days following the last menstrual period. Not infrequently information relative to the size of the uterine cavity, measured at the time of curettage, and to the length of the fetus offered supplementary control material.

#### OBSERVATIONS

Of the 100 specimens examined, 30 had to be discarded as unfavorable for study because of extreme autolysis or scarcity of villi. In the remaining 70, gradual replacement of nucleated by non-nucleated red cells took place rapidly and almost completely during the second and third months of pregnancy. The accompanying graph shows the percentage of immature red corpuscles in the 70 fragments, correlated with the respective ages of the specimens. Before the sixth week the capillaries showed 100 per cent erythroblasts. After the third month normoblasts could be found only in the ratio of 1 to several thousand mature corpuscles. Finer classification of non-nucleated forms into megaloblasts and normoblasts was possible in cases of very early pregnancy.

#### COMMENT

This study brings out the fact that gradual replacement of erythroblasts by mature red cells in chorionic capillaries occurs at a rate definite and constant enough to warrant its forming the basis of a histologic method of determining the age of pregnancy from placental fragments. If all the chorionic corpuscles are nucleated, the pregnancy is probably not older than two months. If more than 1 per cent are nucleated, the age is less than three months. If fewer than 1 per cent of the red cells are immature, pregnancy has passed beyond three months. Abnormal erythropoiesis constitutes the chief factor of error in this method. Otherwise, no special training is required for its use. Small fragments of tissue fixed and stained by the usual technic suffice. With practice, no more than a few minutes need be taken for calculation. Readings can be recorded as actual figures rather than as personal opinions of visual observations. As a consequence of this last feature, enumeration of chorionic red corpuscles in a fetus with normal erythropoiesis assumes a rather independent diagnostic value, especially in cases of early pregnancy in which the history is false or confusing and in which medicolegal problems are involved.

Mention must be made of several implications incidentally brought out by our study. We were early impressed by the fallibility of mea-

surements of the uterine cavity obtained by sounds, as compared with other methods of diagnosing the age of pregnancy.

Of special significance was the relation of our figures to the presumptions commonly accepted by different authors concerning the rate of disappearance of nucleated red cells, their persistence in the fetus in late pregnancy and their presence at birth. Standard textbooks state that normoblasts are found in moderate numbers in infants born at term. Further, they infer that maturation occurs gradually and over an extended period. Such views are held by many. Our observations, in addition to suggesting that the disappearance of erythroblasts takes place rapidly during two months, support the belief of Loos,<sup>4</sup> Hayem,<sup>4</sup> Lewis and Bremer<sup>5</sup> and Starling<sup>6</sup> that nucleated red cells do not occur normally in the new-born or are very rare. In smears from 13 new-born infants, Forkner<sup>7</sup> found nucleated red cells in only 5. From her work, Heath<sup>8</sup> considered low counts (less than from 1 to 2 per cent) physiologic. Counts so low would not be reflected in the total number of red cells (from 100 to 200), which we inspected. Smears from new-born infants were used to check the full term placental specimens. The same infrequency of nucleated red blood cells was noted.

Whether hematologic examination of chorionic capillaries in late pregnancy and at delivery can be used as a means of predicting and prognosticating various pathologic conditions, such as fetal dropsy, icterus gravis neonatorum, anemias of the new-born, prematurity and stillbirth (from fetal erythroblastosis), is an arresting matter for speculation.

#### SUMMARY

In an effort to formulate an additional histologic method of diagnosing the age of pregnancy, 70 placental fragments were examined, and differential counts of the number of nucleated and non-nucleated red cells in the chorionic capillaries at different periods of gestation were made. Replacement of nucleated by non-nucleated forms took place rapidly and almost completely during the second and third months. Therefore, if all the chorionic corpuscles are nucleated, the pregnancy is probably not older than two months. If more than 1 per cent are nucleated, the age is less than three months. If fewer than 1 per cent of the red cells are immature, pregnancy has passed beyond three months.

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# SUBCUTANEOUS NODULES INDUCED BY THE INJECTION OF STREPTOCOCCUS VIRIDANS

SPECIFICITY OF THE LESION AND ORIGIN OF THE POLYBLASTS

LYDIA LUX

MINNEAPOLIS

In a series of reports, Clawson has described the nodule associated with acute rheumatic fever. In 1928 he <sup>1</sup> gave a general description of the inflammatory reaction involved. He described the nodule as a "localized area of inflammation, mostly proliferative in character, found in the loose connective tissue beneath the skin in a limited number of cases of acute rheumatic fever." These nodules, he stated, appear to be similar in morphology to those produced experimentally in rabbits by the injection of *Streptococcus viridans*. In 1929 Clawson <sup>2</sup> commented on the cellular morphology of the nodule and pointed out that the rheumatic nodules should not be regarded as a specific anatomic lesion in the same sense as the tuberculous or syphilitic lesion. In 1930 he <sup>3</sup> described the reaction in normal, immune and hypersensitive animals and concluded that the reaction in hypersensitive animals differed in no way qualitatively from that in normal and immune animals. This view is in accord with that of Maximow. In his work on mesenchymal reactions Maximow <sup>4</sup> said regarding allergic reactions, "As far as one can judge by the still modest accumulations of facts available at present, the differences, as compared with the histogenesis of common inflammation, are quantitative, rather than qualitative." Von Möllendorff <sup>5</sup> agreed that the reaction in a hypersensitive animal differs from that in a normal animal only in degree.

The question of the origin of the free nongranular phagocytic cells in the field of reaction of these lesions is of great importance in this problem. Clawson <sup>3</sup> stated that they are both hematogenous and histogenous in origin. McEwen <sup>6</sup> described a cell derived from the "undifferentiated mesenchyme" of the loose connective tissue, which he thinks

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1. Clawson, B. J.: *Am. J. Path.* **4**:565, 1928.

2. Clawson, B. J.: *Arch. Path.* **8**:654, 1929.

3. Clawson, B. J.: *Arch. Path.* **9**:1141, 1930.

4. Maximow, A.: *Arch. Path.* **4**:557, 1927.

5. von Möllendorff, W.: *Ztschr. f. Zellforsch. u. mikr. Anat.* **6**:61, 1927.

6. McEwen, C.: *J. Exper. Med.* **55**:745, 1932.

is specific for the lesion. This cell will be discussed later. Both Clawson and McEwen referred in detail to conclusions of various earlier workers regarding the origin of these cells; hence it is unnecessary to consider them here.

In this investigation, questions concerning the specificity of the lesion, the behavior of the fibroblasts and the origin of the free phagocytic cells were given special consideration.

#### MATERIAL AND METHODS

The material consisted of nodules removed from hypersensitive adult rabbits that had been killed at intervals of six and twelve hours, one, two, three, four, five, seven and nine days after they had been given multiple injections of *Streptococcus viridans*.

These animals had been made hypersensitive by a subcutaneous injection of about 5 cc. of a heavy suspension of melted agar and streptococci. A large abscess developed at the point of injection. Twelve days later, at the time which had been previously determined to be best for the formation of subcutaneous nodules, the multiple injections mentioned were made into the back of the animals on the side opposite the one with the large abscess.

The organisms were cultured in a broth tube for forty-eight hours, allowed to settle to the bottom of the tube and then decanted. A suspension of the organisms was then made up with 10 cc. of physiologic solution of sodium chloride, and 0.1 cc. of this suspension was used for each of the subcutaneous injections. This dosage proved to be the minimum amount that would produce nodules in normal animals, as previously determined by Clawson.<sup>3</sup>

Several nodules were removed from each animal. A middorsal incision was made, the skin was laid back, and the nodules were found in the tissue adhering to the skin. These nodules appeared as white areas, variable in size and not always easily distinguished from the surrounding tissue.

The removed nodules were fixed for six hours in Zenker's fluid to which a dilute solution of formaldehyde had been added and were washed in running water for twenty-four hours. The paraffin method of embedding was used, and serial sections were made in some instances. Hematoxylin and eosin, methyl green and pyronine, and Maximow's hematoxylin-eosin-azure stains were used on many of the sections, while Dominici's stain was used as a routine procedure.

#### MICROSCOPIC STUDY

*Low Power Magnification.*—The cellular infiltration was diffuse up to the one day stage, while the later stages showed either single or multiple formation of abscesses. The one day stage showed a slight amount of degeneration, while in the four day stage there was an abscess with a central area of necrosis. Abscesses with necrotic centers were present in all of the following stages, but none of these showed evidence of hyalinization.

Edema was marked in the early stages, but after five days little was noted, and it was completely absent in the nine day stage.

Hemorrhage appeared irregularly throughout the series, but it was most marked in the two day stage.

Increased vascularity was noted in all stages, the blood vessels appearing dilated. Migration of cells was more pronounced in the early stages of the reaction than

after the four day stage. The walls of the blood vessels were thickened in many instances but never to the extent of occlusion of the lumens of the vessels.

The fourth day was the earliest stage in which there was definite orientation of fibroblasts suggesting formation of capsules. In the five day stage the number of fibroblasts had increased and the capsule had taken a more definite form. In the last two stages the abscess was completely surrounded by a definite layer of fibroblasts. In all stages there was an increased vascularity in the areas of fibroblastic proliferation.

Throughout this material there could be noted special areas characterized by marked vascularity. Such areas had no obvious relation to the nodules, but the thickening of the walls of the vessels indicated that they were involved in the reaction. In some instances there was marked perivascular lymphocytic infiltration.

*High Power Magnification.*—Edema was one of the earliest responses following the injection of the organisms. It increased progressively in the later stages, reaching a maximum about the fifth day, after which it became less marked. None was noted after the seven day stage.

In all stages there was considerable hemorrhage into the area of reaction. It was most marked, however, in the two day stage.

Cellular infiltration was also an early feature of the reaction. Many polymorphonuclear leukocytes and lymphocytes and a few monocytoïd cells and eosinophils were present in the early stages, but the polymorphonuclear leukocyte was the predominating cell type. There was some degeneration of polymorphonuclear leukocytes in the six hour stage, and this increased in degree as the reaction advanced. Formation of abscesses was noted as early as the one day stage, the abscesses being multiple in many instances. After three days these abscesses were found to have necrotic centers, but there was no evidence of hyalinization. Degeneration of cells, except within the area of the abscess, was no longer a prominent feature after the fifth day.

Comparatively few lymphocytes were present in the six hour stage, but they increased in number in the later stages. Beginning with the two day stage, they showed a tendency to collect around the blood vessels.

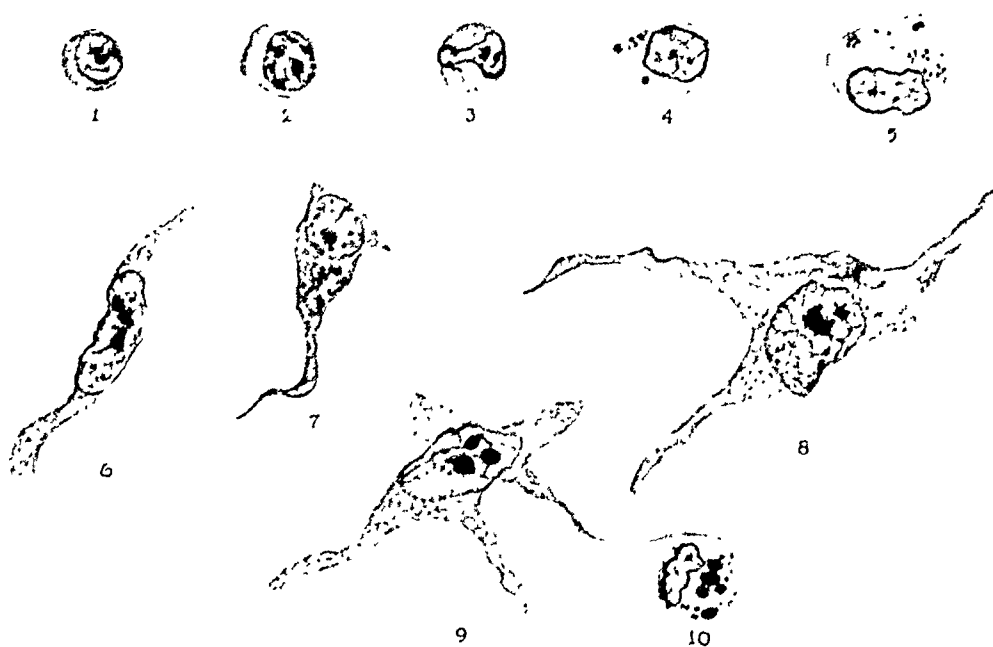
Migration of leukocytes became less prominent as the reaction advanced, but even in the latest stages studied it could be noted.

Few polyblasts were present in the six and twelve hour stages, and only an occasional one was actively phagocytic. After the twelve hour stage there was a marked increase in the number of phagocytic cells, and some were still present in the nine day stage. In the one and two day stages many of the lymphocytes had increased in size, and transitional forms from lymphocyte to polyblast were a conspicuous feature. Even in one microscopic field one could trace all the stages, from small lymphocytes, through hypertrophic intermediate stages, to active phagocytic polyblasts, as is shown in the illustration. The lymphocytes, such as cells 1 and 2, were found in great numbers in some areas. From the transitional forms one could assume that as the lymphocytes enlarged, their cytoplasm became less basophilic, and in many instances vacuolated. The coarse nucleus became less dense in structure and assumed an irregular or indented form. This series of changes is shown in cells 1 to 5. However, many cells retained their original size and were less basophilic but contained some engulfed material. Many of the large phagocytes were loaded with granules from polymorphonuclear leukocytes, nuclear remnants from degenerating leukocytes and erythrocytes.

Among the fibroblasts found in the early stages many were large and swollen in appearance. The outline of the cell was indefinite, and processes were no

longer evident. The cytoplasm was pale and vacuolated, giving the cell a spongy appearance. In many instances the boundary of the cell could not be made out, and the cytoplasm appeared scant or even completely lacking. The nucleus was large and pale. Nucleoli were always present in varying numbers, but usually two were noted. The remainder of the fibroblasts varied widely in appearance, some being practically normal spindle cells while others were quite rounded, with deep basophilic cytoplasm and irregular cell outlines.

After the one day stage the fibroblasts increased in number as the reaction advanced. Although there was some suggestion of concentric orientation of fibroblasts in the three day stage, the fourth day was the first in which there was definite formation of a capsule. In general, in the later stages there was an



The illustration was drawn from sections stained with Dominici's eosin-orange-G-toluidine blue. Cells 1 to 5 are from the subcutaneous tissue of adult rabbits two days after injection. They show the transition of lymphocyte to polyblast. Cells 1 and 2 are small lymphocytes; cells 3 to 5, polyblasts of lymphocytic origin. A single field is represented. Cells 6 to 10 are from subcutaneous tissue of adult rabbits five days after injection; cell 6, a fibroblast drawn from the periphery of the newly forming capsule; cell 7, a fibroblast from a zone between the capsule and a nearby vascular area; cells 8 and 9, fibroblasts from a field close to a vascular area near the capsule; cell 10, a polyblast from this same field.

increase in the thickness of the capsule accompanied by a marked increase in vascularity.

Beginning with the one day stage, where fibroblasts started to increase in number, there was an increase in the variety of types of fibroblasts seen, which reached a maximum on the fifth day. Cells 6 to 9 were drawn from this stage to show the extreme variation in form. In general, the small forms had a deeply



basophilic cytoplasm; some were almost round. They assumed the flattened form as one approached the area in which the capsule was being laid down. Cells 6 to 9 illustrate some of the more extreme forms. These resembled in certain ways the damaged cells described in the early stages. They had a pale vacuolated cytoplasm, but they were more branched and had a more definite cell boundary than the damaged cells. Furthermore, the nucleus was not as pale. It was not possible to follow the damaged cells through the three and four day stages and to establish with certainty their relation with the extreme forms seen in the fifth day.

The fibroblasts at no time proved to be phagocytic, though they were found in areas where degenerating leukocytes and many active phagocytic polyblasts were present, as is shown by cells 9 and 10 which were in the relation depicted in the illustration.

The capsule completely surrounded the abscess after the four day stage. At the junction of the abscess with the inner surface of the capsule there was a considerable number of phagocytic polyblasts present, and at several points the fibroblasts were beginning to penetrate the central necrotic mass. This penetration by the fibroblasts was particularly marked in the seven and nine day stages.

In all stages of the reaction there was a dilatation of blood vessels. There was also a marked thickening of the endothelium, and in the early stages there was some suggestion that free cells were being liberated into the lumen. In some places it appeared that the vessel had become entirely disorganized, leaving a mass of fibroblast-like cells, the exact origin of any one of which could not be determined. This was especially noted in the two, three and four day stages.

#### COMMENT

The specificity of rheumatic nodules has been a much discussed question. In 1904 Aschoff<sup>7</sup> described this type of inflammatory reaction as specific for acute rheumatic fever. Sometime later Coombs,<sup>8</sup> in his work on rheumatic infections, also stated that this type of lesion was specific for rheumatic fever. In 1914 Thalheimer and Rothschild<sup>9</sup> confirmed that opinion when they reported that in rheumatic myocarditis foci termed "submiliary nodules of Aschoff are present which are characteristic of rheumatic infections."

Contrary to this point of view, Clawson<sup>2</sup> in his report on the Aschoff nodule found that rheumatic nodules cannot be considered as characteristic of rheumatic fever. He stated that they are more commonly found in association with acute rheumatic fever, but that they are also found in cases of nonrheumatic infectious diseases. He did not consider the histologic picture distinctive for this type of lesion.

It has already been stated that Clawson<sup>1</sup> concluded that the experimental nodules were similar in histology to the nodules found in subcutaneous tissues in cases of acute rheumatic fever.

In the material studied here, the early stage was essentially exudative with a subsequent breakdown of the central part of the area involved.

7. Aschoff, L.: *Verhandl. d. deutsch. path. Gesellsch.* 8:46, 1904.

8. Coombs, C.: *Brit. M. J.* 2:1513, 1907.

9. Thalheimer, W., and Rothschild, M. A.: *J. Exper. Med.* 19:417, 1914.

The abscess was then surrounded by a reaction field occupied by free ameboid phagocytic cells of various sizes. In the later stages emigration of hematogenous cells was still taking place to some extent. The cells of the lymphoid series were becoming more numerous in relation to the granulocytes.

The fibroblasts were involved from the start. They soon lost their processes, increased in size, and in general showed changes that appeared to be degenerative. They began to increase in number after the one day stage, and this increase was particularly marked on the third day when they began to participate in the formation of a capsule. From this point on the picture was one of fibroblastic proliferation. Obviously there was overlapping of these processes.

This general description as well as the detailed microscopic study on which it is based shows that the course of events here corresponded closely with that well established for inflammatory reactions in general. These observations therefore in no way support the contention that the lesion shows a characteristic histologic picture.

#### ORIGIN OF FREE NONGRANULAR PHAGOCYTIC CELLS

Clawson<sup>3</sup> found evidence that the phagocytic cells in these nodules were both hematogenous and histogenous.

McEwen<sup>6</sup> studied the large irregularly shaped cells found in scrapings of subcutaneous nodules removed from patients with rheumatic fever. From the results of supravital staining he concluded that these cells took origin from the undifferentiated mesenchyme of the loose connective tissue. He based his conclusion on the fact that the "granuloma cells" did not take up neutral red in supravital stained preparations, whereas other cells took up various amounts of dye according to their nature. As additional evidence that these cells are undifferentiated he points out that they exhibit developmental tendencies; that is, a limited number of them take up particles of carbon. He believes that certain transitional forms indicate that they may be transformed into fibrocytes.

Regarding the origin of free nongranular cells in nonspecific inflammatory processes, there are two schools of thought. As early as 1905 Maximow<sup>10</sup> described septic and aseptic inflammatory reactions and stated that the origin of polyblasts from hematogenous cells was evident, but that fibroblasts contributed in no way to the phagocytic cells present. The fibroblasts in the immediate field he described as showing degenerative changes with the nucleus shrunken in appearance and a much vacuolated cytoplasm. The fibroblasts more distant from the field of reaction were forced apart but not altered.

10. Maximow, A.: Beitr. z. path. Anat. u. z. allg. Path. **38**:301, 1905.

In 1927, Maximow<sup>4</sup> discussed in detail the cells involved in inflammatory reactions. He stated that the polyblasts originated from lymphocytes and monocytes and to a lesser degree from the histiocytes of the connective tissue. He found no evidence for the origin of ameboid phagocytic cells from endothelium of blood vessels and cited the work of Lang<sup>11</sup> and Stilwell<sup>12</sup> as corroborating this view. He claimed that the endothelium merely formed capillary sprouts and that in inflammation and culture of tissue some of its cells became fibroblasts. In all of his work, Maximow found no evidence of fibroblasts becoming phagocytic polyblasts. He noted,<sup>13</sup> however, in his cultures of lymph nodes inoculated with tubercle bacilli, an "embryonic transformation" of the fibroblasts which he considered as a "specific" reaction of these cells to the tubercle bacillus.

Two years later when Maximow<sup>14</sup> repeated the work of von Mölendorff with subcutaneous injections of trypan blue, he again found that most of the polyblasts in the field of reaction took origin from hematogenous lymphocytes and monocytes, and that after two days phagocytes derived from lymphocytes could not be distinguished from those that originated from monocytes.

In 1925 Maximow<sup>15</sup> postulated the "undifferentiated mesenchyme cells" of connective tissue, which morphologically appear to be fibroblasts but differ physiologically, since they are presumed to have practically unlimited developmental potencies. The undifferentiated mesenchyme cells in contrast to histiocytes do not store colloidal dyes. These cells are found particularly along blood vessels.

Bloom<sup>16</sup> injected a suspension of *Bacterium monocytogenes* into a rabbit subcutaneously. An abscess developed which was similar to reactions due to other inflammatory stimuli. He noted that the phagocytes of the abscess were practically exclusively of hematogenous origin. He stated that "in none of these inflamed tissues was there any evidence in favor of the special leukocytes and exudative mononuclear phagocytes developing either from fibroblasts or from vascular endothelium."

Michels and Globus,<sup>17</sup> in their descriptions of infiltrations of round cells in the central nervous system, stated that in dementia paralytica and in encephalitis the vast majority of exudate cells originated from emigrated lymphocytes and monocytes and that a small number were "homoplastic derivatives of preexistent or previously extravasated

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11. Lang, F.: *Arch. Path.* **1**:41, 1926.

12. Stilwell, F.: *Folia haemat.* **33**:81, 1926.

13. Maximow, A.: *J. Infect. Dis.* **34**:549, 1924.

14. Maximow, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **82**:1, 1929.

15. Maximow, A.: *J. Infect. Dis.* **37**:418, 1925.

16. Bloom, W.: *Arch. Path.* **6**:995, 1928.

17. Michels and Globus: *Arch. Path.* **4**:692, 1927; **8**:371, 1929.

lymphoid cells." Formation of exudate cells from the so-called adventitial cells of Marchand or any other fixed connective tissue cells was not noted in their work. In all instances, even when perivascular infiltration was maximal and connective tissue was hypertrophic, they found no rounding of fibroblasts and no amitosis. '

The point of special interest in these contributions is the fact that they present no evidence in favor of the origin of polyblasts from fibroblasts.

On the other side of this question we find that as early as 1911, Downey<sup>18</sup> called attention to the participation of fibroblasts in the formation of plasma cells. In his work<sup>19</sup> on the structure and origin of lymph sinuses of mammalian lymph nodes he pointed out that fibroblasts ordinarily reacted slowly to vital dyes and foreign substances. However, fibroblasts of the omentum, which formed an exception to this rule, reacted vigorously to vital dyes and foreign substances and readily became free cells which could differentiate into various types of lymphoid cells.

Lindsey,<sup>20</sup> under the direction of Downey, worked on reactions of the subcutaneous tissue to experimental tuberculosis in the guinea-pig. She regarded the fibroblasts as "relatively undifferentiated cells closely related to mesenchyme." She claimed that these cells were transformed into exudate cells, including clasmatoocytes, monocytes, lymphocytes, polyblasts and epithelioid cells. She found monocytoïd cells originating locally from fibroblasts, and these represented intermediate stages in the formation of epithelioid cells. Similar investigations were continued by Downey's student, Ekola,<sup>21</sup> who worked on reactions of subcutaneous tissue to foreign substances. She found transitional stages on which she based the conclusion that fibroblasts gave rise to polyblasts and clasmatoocytes. She also found evidence for the origin of polyblasts clasmatoocytes and lymphocytes.

Watson,<sup>22</sup> also working under Downey's direction, described a case of histoplasmosis in which he made special reference to the origin of phagocytic cells. In the liver these cells took origin from small lymphocytes, reticulo-endothelial cells of the liver sinusoids, and rarely from fibroblasts. He found that the macrophages in the lymph nodes were from two sources, the lymphocytes and reticular cells, while those in the lung came from lymphocytes, alveolar epithelial cells and adventitial histiocytes from capillaries of the lung.

18. Downey, H.: *Folia haemat. Arch.* **11**:271, 1911.

19. Downey, H.: *Haematologica* **3**:431, 1922.

20. Lindsey, M. L.: *Am. Rev. Tuberc.* **19**:615, 1929.

21. Ekola, M. W.: *Folia haemat.* **43**:4, 1931.

22. Watson, C. J.: *Folia haemat.* **37**:70, 1928.

Any discussion of the origin of macrophages from fibroblasts must take into consideration the opinions of von Möllendorff.<sup>23</sup> From his investigation of inflammatory reactions induced by subcutaneous injections of trypan blue in mice and rabbits, he concluded that polyblasts were only in part hematogenous. He maintained that fibroblasts played an important rôle in the production of phagocytes and granulocytes in inflammation. The fibrocytes of loose connective tissue he considered as part of a diffuse syncytium which when stimulated gave rise to many large ameboid mononuclear cells. He regarded the adventitial cells of Marchand as a stimulated part of the net in the region of the vessels. The first changes noted in his experiments were granulation and vacuolation of some of the fibroblasts with a contraction of the cell and nucleus and a complete separation from the net as free cells. These free cells, for the most part, even as early as twenty-eight hours after injection, became round cells with polymorphous nuclei and then degenerated.

One year later, while working with normal and hypersensitive animals, von Möllendorff<sup>24</sup> injected serum subcutaneously and found that after only a few hours one could distinguish transitional stages between fibroblasts and large basophilic round cells that finally became granulocytes and then degenerated.

Maximow<sup>14</sup> in repeating the work of von Möllendorff found no proof for the origin of free phagocytic cells from fibroblasts.

Lindsey<sup>20</sup> agreed in part with von Möllendorff when she stated that fibroblasts of the subcutaneous tissue into which tubercle bacilli were injected gave rise to exudate cells. However, she found no evidence for the formation of polymorphonuclear leukocytes from fibroblasts. The nuclei of many polyblasts became lobulated, and these, she claimed, looked like pyknotic polymorphonuclears, the granules of which had disappeared.

Ekola<sup>21</sup> also concluded that fibroblasts gave rise to polyblasts and clasmotocytes. In many instances the intermediate forms were vacuolated and were active phagocytic cells. However, she found no evidence that granulocytes developed from connective tissue cells as was claimed by von Möllendorff.

Bloom,<sup>25</sup> in his excellent summary on cellular relationships in general, commented on the origin of macrophages from fibroblasts. While he stated that he does not think that fibroblasts are an important source of macrophages in inflammatory reactions in general, he did not deny the possibility that the change can take place.

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23. von Möllendorff, W., and von Möllendorff, M.: *Ztschr. f. Zellforsch. u. mikr. Anat.* **3**:503, 1926.

24. von Möllendorff, W.: *München. med. Wchnschr.* **74**:135, 1927.

25. Bloom, W.: *Arch. f. exper. Zellforsch.* **11**:145, 1931.

There are a number of reports from workers on tissue cultures, beginning with the report of Carrel<sup>26</sup> in which he stated that twice in fourteen years he observed in the periphery of his cultures of embryonic heart muscle a spontaneous change of fibroblasts to free ameboid cells.

Fischer,<sup>27</sup> in 1926, in his cultures derived from chick heart which he regarded as pure fibroblasts, found that these fibroblasts became ameboid macrophages that differed from fibroblasts morphologically and physiologically. These cells were different from those found in dying and degenerating cultures.

Parker<sup>28</sup> found a transformation from fibroblast to macrophage in his culture of fibroblasts derived from heart and skeletal muscle. Only a limited number of his cultures showed this transformation, which seemed to take place at some critical period in the life of the culture in which degeneration was taking place at a relatively slow rate. He quoted Ephrussi and Hugues<sup>29</sup> as having noted this same transformation.

The material studied in this investigation supports the contention that the fibroblasts in no way contribute to the formation of phagocytic cells. The change in the fibroblasts as evidenced early was essentially a shortening or loss of processes. The outline of the cell was irregular and poorly defined, and the cytoplasm was pale and vacuolated. In many instances the cytoplasm was scant and at times even completely lacking. The nuclei were large and vesicular and were often found in groups when no outline of cells was evident. At no time were these cells phagocytic, nor could intermediate forms be found to suggest any relation to the phagocytic polyblasts present in the material.

When fibroblastic proliferation began the picture changed and there was a sudden marked increase in fibroblasts. At no time was there a great number of mitotic figures or evidence of amitosis. There was some suggestion that polyblasts were being transformed into fibroblasts, but no satisfactory series of intermediate stages could be found.

The origin of polyblasts from hematogenous cells was evident. Early in the reaction transitional stages between the various lymphoid cells which showed practically no phagocytic tendencies and polyblastic phagocytes of various sizes could be readily followed, as is indicated by cells 1 to 5 in the illustration. The transitional forms show a cytoplasm that is less basophilic and more vacuolated, while the nucleus is irregular in form and of a less dense structure.

The picture here is identical with that described by the group which derives the polyblasts from hematogenous cells. That fibroblasts undergo marked changes is obvious, but there is nothing in this material

26. Carrel, A., and Ebeling, A. H.: *J. Exper. Med.* **44**:261, 1926.

27. Fischer, A.: *Arch. f. exper. Zellforsch.* **3**:345, 1926.

28. Parker, R.: *J. Exper. Med.* **55**:713, 1932.

29. Ephrussi, B., and Hugues, Y.: *Compt. rend. Soc. de biol.* **105**:697, 1930.

to justify the conclusion that these changes should be interpreted as leading to the formation of phagocytic cells.

Special note should be taken of the work of Lindsey and Ekola in their confirmation of von Möllendorff on this point. And the opinions of those working with culture of tissue, in which less complex conditions render errors of interpretation less probable, have also been cited in favor of the fibroblastic origin of phagocytes.

While the particular materials and methods used in this investigation entirely failed to show the transformation of fibroblasts into phagocytes, I am willing to admit that under other circumstances this transformation may take place.

#### CONCLUSIONS

The nodules studied revealed a nonspecific inflammatory reaction.

The lymphoid cells of the exudate constitute the chief source of phagocytic cells seen in these lesions.

There is no evidence that fibroblasts are transformed into phagocytic cells.

# General Review

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## ORIGIN AND NATURE OF THE LESION OF SYMPATHETIC OPHTHALMIA

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BROOKLYN

Even the laity is aware of the fact that a person sustaining an injury to one eye is liable to lose the vision in the uninjured eye by virtue of some mysterious process called sympathy or sympathetic disease. Many physicians, while generally conversant with the clinical aspects of this phenomenon, are perhaps as unfamiliar as are the laymen with the pathologic process involved. It is with this thought in mind that I am presenting a review of the knowledge of this unusual disease which has furnished the basis for an enormous volume of philosophic discussion and intriguing although not conclusive research.

### THE TYPICAL LESION

A small percentage of enucleated eyes show, on histologic examination, a picture which has come to be looked on as practically pathognomonic of sympathetic ophthalmia.

In sections of such eyes one finds an inflammatory lesion of the granulomatous type involving chiefly the uveal tissue with little involvement of other structures. There is rather diffuse or focal round cell infiltration of the iris, ciliary body and choroid, the degree of infiltration being variable in the different structures but tending to involve the entire uvea. Among the round cells are found nests of closely packed epithelioid cells which frequently contain giant cells of the Langhans type. The epithelioid collections closely resemble epithelioid tubercles except for the absence of caseation.

A striking characteristic is the constancy with which the process is confined to the uvea. Invasion of the sclera is limited to the region of the emissaria, where mantles of round or epithelioid cells may be found surrounding the perforating vessels and nerves. Occasionally there is extension into the optic nerve head, while only rarely is there any invasion of the retina.

Omitting, for the moment, consideration of minor features, a picture such as I have described could be considered fairly diagnostic of

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this disease entity. If, in addition, it were known that the sections were from an eye which had sustained a perforating wound in the ciliary region and that, prior to enucleation, the other (uninjured) eye had begun to show signs of inflammation, a diagnosis of sympathetic ophthalmia could be made with certainty.

#### DEFINITIONS

*Sympathetic Ophthalmia.*—Prior to the anatomic studies of Schirmer<sup>1</sup> and Fuchs<sup>2</sup> and his school, the term sympathetic ophthalmia was understood to refer only to the disease in the uninjured eye. The lesion in the injured eye was thought to be an ordinary traumatic endophthalmitis which in some way was communicated to the opposite eye. These investigators showed that an anatomically well defined and apparently specific form of uveitis could be found in the injured eye as well as in the eye suffering in sympathy. This process was designated by Fuchs as sympathetic inflammation.

The tendency, still evident in the literature, to designate as sympathetic ophthalmia only the process in the eye which is secondarily involved is perhaps in part reminiscent of the older concept and in part due to the lexicologic force of the word sympathetic. From a pathologist's point of view, it seems advisable to employ the term "sympathetic ophthalmia" (in the sense of Fuch's sympathetic inflammation) to indicate the characteristic lesion of the disease and not the extent of involvement of the susceptible (uveal) tissue.

With this preamble I would broadly define sympathetic ophthalmia as a granulomatous lesion of the eye characterized chiefly by its tendency to involve the uveal tissue of both eyes.

*Exciting<sup>3</sup> Eye.*—Assuming for the present that the disease begins first in one eye, which in most instances has sustained a perforating wound (nonsurgical or surgical) in the ciliary region, this eye is variously described as the "primary" eye, the "sympathogenic eye" or, as I shall refer to it, the exciting eye.

*Sympathizing<sup>4</sup> Eye.*—The eye subsequently involved, sometimes called the "secondary" eye, will be referred to as the sympathizing eye.

Such terms as sympathetic iritis, sympathetic choroiditis and sympathetic scleritis, frequently encountered in the literature, are employed not to indicate separate disease entities but to designate the involvement

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1. Schirmer, O.: (a) Arch. f. Ophth. **38**:4, 1892; (b) in Graefe-Saemisch: Handbuch der gesamten Augenheilkunde, Leipzig, Wilhelm Engelmann, 1900, vol. 6, p. 2.

2. Fuchs, E.: (a) Arch. f. Ophth. **61**:365, 1905; (b) **70**:465, 1909.

3. Sympathisierende, sympathisant, simpatizzante.

4. Sympathisierte, sympathisé, simpatizzato.

of the various portions of the eye in the disease process. When the process is almost exclusively limited to, or unusually prominent in, one or another of these structures, such a term is more accurately descriptive than the general term, sympathetic ophthalmia.

### HISTORICAL REVIEW

Concerning the early history of sympathetic ophthalmia, one might best refer to Parsons' <sup>5</sup> "Pathology of the Eye," from which, because of its relative inaccessibility, the following passages are quoted:

The earliest reference to sympathetic ophthalmia is found in the oldest German text-book of ophthalmology by Bartisch (1583), who remarks that in cases of injury to one eye "the other good eye is besides also in great danger." Le Dran (1741) says: *Si comme aux abcès qui se font ailleurs on attend que le pus soit fait le malade pourra perdre la vue par l'inflammation qui se communiquera à l'autre oeil le long du nerf optique.* Two undoubted cases are recorded by Demours (1818). Wardrop (1819) reported a case of sympathetic iritis briefly, and mentioned that veterinary surgeons had noticed destruction of the second eye in horses, and that it might be avoided by destruction of the first eye by lime (cf. Barton, Crompton). Lawrence (1833) recorded several cases. The true history of sympathetic ophthalmia may be said, however, really to have commenced with the masterly description and imaginative insight of William Mackenzie (1835).

Sympathetic ophthalmia was first mentioned under this designation in Germany by v. Ammon (1838). Neither he nor Himly (1843) appear [sic] to have been conversant with Mackenzie's work; his name is not mentioned and the descriptions are much less accurate and complete.

The next notable contribution to our knowledge of the disease was made by Augustin Prichard (1851), of Bristol, who first proved the efficacy of excision of the exciting eye. This treatment was received with distrust, even by v. Graefe, who preferred iridectomy, and finding this useless, resorted to the production of panophthalmitis by passing a thread through the eye; he mentions optico-ciliary neurotomy, but does not appear to have employed it. v. Graefe later recognized the value of excision of the exciting eye, and advanced the opinion (1866) that inflammation of the uveal tract was *sine qua non* to sympathetic inflammation. At the Ophthalmological Congress at Heidelberg in 1863 George Critchett contributed important additions to our knowledge of the clinical picture of the disease, v. Graefe distinguished between the more malignant (plastic) and less grave (serous) forms of sympathetic iritis, and Donders adduced evidence of a sympathetic neurosis which is promptly cured by excision of the exciting eye.

The more recent history of the disease concerns largely the development of modern concepts of the nature of the pathologic process and investigations into its etiology and pathogenesis. These will be discussed later.

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5. Parsons, J. H.: *The Pathology of the Eye*, New York, G. P. Putnam's Sons, 1908.

## LESIONS THAT MAY GIVE RISE TO SYMPATHETIC OPHTHALMIA

Most cases of sympathetic ophthalmia occur following traumatic perforating wounds in the ciliary region, which, in this sense, has become known as the dangerous zone. Less frequently the disease develops following operative procedures such as iridectomy or cataract extraction. It is usually noted that prompt and uneventful healing of the wounds has been prevented by complications such as traumatic cyclitis or incarceration of uveal tissue or lens capsule between the edges of the wound. The fact that wounds in the ciliary region are prone to such complications may account for the peculiar significance of the location of wounds (surgical or nonsurgical) in this regard.

Frankly infected wounds which lead to purulent endophthalmitis or panophthalmitis are unlikely to be followed by sympathetic ophthalmia, a fact for which no satisfactory explanation has been advanced. Occasional instances of sympathetic ophthalmia following suppurative lesions were cited from the literature by Schirmer, Peters and others and were referred to in an article by Lamb<sup>6</sup> in which he recorded a case of this kind.

The disease rarely follows perforating corneal ulcer, perhaps for the reason that purulent panophthalmitis is the common sequela of such a lesion.

There remains a small but important group of cases in which sympathetic ophthalmia has been reported as following nonperforating traumatic lesions, or intra-ocular tumors (usually melanomas) with or without perforation of the globe. They have given rise to a great deal of discussion, and the decisions to accept or reject individual cases in the group as instances of sympathetic ophthalmia seem to depend in part on the leanings of the reviewers with regard to the nature of the immediate causative agent and its mode of entrance into the eye. Parsons<sup>5</sup> suggested that "a minute wound of the conjunctiva or an abrasion through which exogenous infection might take place most probably explains the few doubtful cases [of subconjunctival rupture of the globe] in which sympathetic ophthalmia may have occurred."

Lamb,<sup>7</sup> in 1932, reported what seems to be an undoubtable instance of sympathetic ophthalmia following a nonperforating injury. The cornea of one eye had been slightly abraded by the blow of a stick, but no deep wound could be found. The second eye became inflamed ten weeks after the injury and, in spite of enucleation of the injured

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6. Lamb, H. D.: *Tr. Am. Ophth. Soc.* **29**:288, 1931.

7. Lamb, D. H.: *Arch. Ophth.* **7**:97, 1932.

eye, it became blind and shrunken. The diagnosis was substantiated by histologic study of the enucleated (injured) eye. Lamb reviews cases of this nature reported by Kitamura, Botteri, Meller, Weigelin and Guillery, in each of which, in his opinion, there had been either a possibility of doubt as to the correctness of the diagnosis or some complicating factor such as operative intervention.

A similar case reported by Delaney,<sup>8</sup> in 1931, presented a typical clinical picture of sympathetic ophthalmia developing nine months after injury to one eye by a snowball. The enucleated injured eye failed, however, to show the typical histologic features of the disease, a fact which by no means disproves the authenticity of the case, as will be explained subsequently.

Leber and Krahnstöver<sup>9</sup> considered there was not sufficient evidence to show that sympathetic ophthalmia is ever set up by sarcoma of the choroid, though it might follow perforation of the globe through operative intervention.

Mention is usually made of extensive necrosis of the tumor tissue in these cases with the implication that it is the necrosis which has some causal relationship to the sympathetic lesion. Lamb,<sup>6</sup> in reporting a case of this type, refers to similar cases recorded by Fuchs, Meller and Reis.

The status of these cases will likely remain problematic until the *causa causans* of sympathetic ophthalmia is definitely proved.

The time interval between injury of the one eye and the appearance of signs and symptoms of a sympathizing inflammatory process in the other eye is extremely variable. The shortest interval is fourteen days. More often it is from four to twelve weeks, after which time the likelihood of development of the disease becomes progressively less. The longest interval recorded is forty-eight years (Smith<sup>10</sup>).

According to Parsons,<sup>5</sup> in nearly all cases in which the interval has been long the injured eye has become irritable and inflamed shortly before the onset of the sympathizing process in the other eye. "This may be spontaneous or due to trauma, often scarcely appreciable." Conversely, as stated by Smith, "when an injured eye has been free from inflammatory reaction for months or years, the subsequent appearance of inflammation in the second eye without a history of flare-ups or attacks of pain in the injured eye should be regarded as an independent condition probably of constitutional origin and therefore not directly related to sympathetic ophthalmia."

8. Delaney, J. H.: Arch. Ophth. 5:781, 1931.

9. Leber, T., and Krahnstöver, A.: Arch. f. Ophth. 45:164, 1898.

10. Smith, J. W.: Arch. Ophth. 2:169, 1929.

The frequent finding of bone in the choroid or in cyclitic membranes of shrunken exciting eyes is regarded as a sequela of phthisis bulbi and not of particular significance in sympathetic ophthalmia.

Obviously such data as the time of injury of one eye and the time of appearance of inflammation in the other eye furnish no indication of the time of onset of the specific disease in the exciting eye, and no other criteria are available by which this can be accurately determined. The specific process is always more or less completely obscured from clinical observation by the direct results of trauma or by an associated traumatic endophthalmitis. Similarly, the histologic features of an early sympathetic ophthalmia in the exciting eye may either be masked by nonspecific changes or be unrecognized because of the unfamiliarity with the earliest phases of the process.

In a clinically suggestive case, in which a traumatized eye has been enucleated as a prophylactic measure, the earliest histologic manifestations of sympathetic ophthalmia may indeed be present but not recognizable as such. Conversely, a histologically suggestive picture in an enucleated eye cannot establish the diagnosis in the absence of a sufficiently characteristic clinical history. The diagnosis in either instance is only presumptive.

The rate of development of the process in the exciting eye is evidently extremely variable. In a case reported by Kümmell<sup>11</sup> there were found only isolated lymphocytic foci in spite of the fact that the disease in the sympathizing eye had continued for a year. Ginsberg<sup>12</sup> suggested that in such a case "one is to assume that the process is in the act of retrogression." It is not uncommon, on the other hand, to find a well developed characteristic picture a few weeks after injury, as in one of my cases in which enucleation followed immediately on the onset of signs of involvement of the other eye. In this instance it seems evident that the granulomatous lesion in the exciting eye must have developed rapidly and anticipated, at least by several days, the onset of the process in the sympathizing eye.

#### HISTOLOGY

The histologic picture of sympathetic ophthalmia as seen in the exciting eye is always more or less complicated by the effects of injury per se or by reaction to a resulting nonspecific infection. Such exudative and reparative processes as might follow any perforating wound, and

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11. Kümmell, R.: *Arch. f. Augenh.* **86**:143, 1920.

12. Ginsberg, Sigmund, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1928, vol. 2, pt. 1.

particularly plastic exudation, are not, according to Fuchs,<sup>2a</sup> of the nature of sympathetic ophthalmia. This view is in opposition to the earlier views of Schirmer and his pupil Ruge,<sup>13</sup> who believed that plastic exudate is a characteristic, but not essential, feature of sympathetic ophthalmia. The sympathizing eye, on the other hand, is likely to show the uncomplicated picture of this apparently specific form of uveitis. The observations of Milles,<sup>14</sup> Deutschmann,<sup>15</sup> Zimmermann,<sup>16</sup> Grunert,<sup>17</sup> Schirmer,<sup>1b</sup> Welt,<sup>18</sup> Asayama,<sup>19</sup> Blascheck,<sup>20</sup> Lenz,<sup>21</sup> Pöllot,<sup>22</sup> Meller,<sup>23</sup> and others showed the process in the sympathizing eye to be otherwise essentially the same as that seen in the exciting eye, which is usually the one available for histologic examination.

The most characteristic involvement is seen in the choroid. Round cell infiltration appears first in the middle and outer layers, leaving the choriocapillaris, at least for a time, free from change. The round cells, in the earlier stages, are found in irregularly placed groups but may become so numerous as to form a dense, confluent layer throughout the choroid, even causing a degree of thickening which is grossly evident on section. Among the round cells and sometimes in round cell-free areas there are found nests of epithelioid cells which, under low magnification, appear in the stained sections as pale patches contrasting sharply with the deeply staining masses of round cells. Particularly when they contain giant cells these collections of epithelioid cells closely resemble epithelioid tubercles. The round cells are usually composed predominantly of lymphocytes with an admixture of plasma cells, while neutrophilic polymorphonuclears and eosinophils may also be found. The choroidal involvement is seldom less severe than that in other parts of the uvea and often represents almost the entire extent of the process (Fuchs<sup>2</sup>).

In the iris the degree of involvement is extremely variable. The infiltration usually starts near the posterior surface, often behind the dilator and sphincter muscles which may become widely separated from the pigment epithelium by this infiltration. Epithelioid cells appear later

13. Ruge, S.: *Arch. f. Ophth.* **57**:401, 1904; **65**:135, 1907.
14. Milles, W. J.: *Roy. Lond. Ophth. Hosp. Rep.* **6**:48, 1886.
15. Deutschmann, R. H.: *Beitr. z. Augenh.* **1**:850, 1893.
16. Zimmermann, W.: *Arch. f. Ophth.* **42**:39, 1896.
17. Grunert, K.: *Klin. Monatsbl. f. Augenh.* **38**:1, 1900; **39**:833, 1901.
18. Welt: *Rev. méd. de la Suisse Rom.* **22**:408, 1902.
19. Asayama, J.: *Arch. f. Ophth.* **54**:444, 1902.
20. Blascheck, A.: *Ztschr. f. Augenh.* **9**:434, 1903.
21. Lenz, G.: *Klin. Monatsbl. f. Augenh.* **45**:468, 1907.
22. Pöllot, W.: *Arch. f. Ophth.* **81**:264, 1912.
23. Meller, J.: *Arch. f. Ophth.* **101**:122, 1920.

than the round cells and gradually replace them. The granulomatous process may involve the entire iris and extend backward into any exudate present in the posterior chamber. Giant cells are said to be less numerous here than in other portions of the uvea (Parsons<sup>5</sup>), although in one of my cases they were very numerous. "The granulomatous tissue, in contradistinction to tuberculosis, never grows out over the free surface of the iris" (Ginsberg<sup>12</sup>).

The ciliary body always shows infiltration, sometimes more marked than in the choroid. It begins in the loose layer of connective tissue and seldom involves, to any great extent, the ciliary muscle. Epithelioid masses may produce marked thickening of both ciliary and orbicular portions. Giant cells are often conspicuous in this region. I have repeatedly found a quite orderly row of them in the neighborhood of the elastic lamina.

In both the iris and the ciliary body the pigment epithelium becomes disorganized, and the cells proliferate and migrate.

The nonpigmented ciliary epithelium is more likely to remain intact, but eventually may become much disturbed. The cells proliferate and wander out along the planes of the zonula, and among them are found leukocytes and epithelioid cells. The epithelial cells may even contribute to the building of collections of epithelioid cells (Ginsberg<sup>12</sup>).

Samuels,<sup>24</sup> in a recent study of the conditions existing at the site of injury in cases of sympathetic ophthalmia, found the specific infiltration in the neighborhood of the wound in a high percentage of cases. In a series of eighty-eight cases in which suitable sections were available there were two cases in which the infiltration was limited to the site of the wound. Nine cases showed more involvement here than elsewhere. In two cases there was no specific involvement at the site of the wound, and in ten it was less marked here than elsewhere in the uvea. In the remaining fifty-eight cases there was "about the same amount of infiltration in the various divisions of the uvea," i. e., not particularly more marked or less marked at the site of the wound. Regarding the relative age of the process in the various parts of the uvea, the author found that generally the infiltration was older in the anterior segment as indicated by the more frequent occurrence here of giant cells and epithelioid cells, while posteriorly the infiltration consisted only of nodules of lymphocytes. Samuel's conclusions regarding the significance of these findings will be discussed elsewhere.

I have mentioned the remarkable predilection of the disease for uveal tissue and its tendency to spare the other ocular structures. This is indeed striking when one compares the lesion with one of a more

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24. Samuels, B.: *Tr. Am. Ophth. Soc.* **30**:253, 1932.

invasive character such as frank tuberculosis or syphilis. On closer analysis, however, the apparently sharp delimitation of the process becomes less impressive. The granulomatous lesion commonly extends into the sclera along the sheaths of uveal tissue which accompany the perforating vessels and nerves (the so-called emissary channels) and from these foci there proceeds more or less marked infiltration of the scleral tissue proper. In this way the process may extend quite through the sclera but seldom invades, to any appreciable extent, the adjacent orbital tissue.

The optic nerve head commonly shows some infiltration. The retina is seldom involved to a marked degree. In one of Meller's<sup>25</sup> cases there were large tumor-like proliferations, but such cases are evidently rare. More commonly, there are only small, occasional, epithelioid foci growing through from the choroid. Fuchs<sup>2a</sup> noted involvement of the retina in one case of a series of thirty-five. Friedenwald<sup>26</sup> found epithelioid foci in the retina in ten of a series of twenty cases of sympathetic ophthalmia. In one case, in which there was a retinal detachment of long standing, I found similar foci among the folds of the retina. Dalén<sup>27</sup> described cellular nodules beneath the retina on an apparently intact lamina vitrea (Dalén foci) which, according to Fuchs,<sup>2a</sup> were composed exclusively of derivatives of the pigment epithelium. Ginsberg<sup>12</sup> commented, in effect, that while such proliferations of the pigment epithelium occur, perhaps they represent the first reaction in processes that precede the wandering through of the granulomatous lesion. It is apparently not uncommon to find such extensions of the choroidal lesions elevating the retina or growing into postretinal exudate.

The epithelioid cells everywhere show a marked tendency to phagocytose uveal pigment, a fact which is perhaps paralleled by Friedenwald's<sup>26</sup> recent observations regarding the cutaneous lesion at the site of intracutaneous injections of pigment, which will be discussed later.

Necrosis is rather rare in the lesion of sympathetic ophthalmia and, according to Meller,<sup>28</sup> differs from the caseation of tuberculosis in that it occurs in streaks or channels among and not in the center of the collections of epithelioid cells. Samuels<sup>24</sup> mentioned that "that liquefaction that is sometimes seen in streaks in large masses of specific infiltration is not to be confounded with the caseation of tuberculosis."

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25. Meller, J.: *Arch. f. Ophth.* **89**:437, 1914.

26. Friedenwald, J. S., in discussion on Woods and Little.<sup>51d</sup>

27. Dalén, A.: *Mitt. a. d. Augenklin. d. Carolin. med.-chir. Inst. zu Stockholm* **6**:1, 1904.

28. Meller, J.: *Arch. f. Ophth.* **89**:248, 1914.



## OUTLINE OF CLINICAL FEATURES

The more purely clinical features of the disease will be dealt with only briefly. Recognition of the clinical picture of sympathetic ophthalmia in the exciting eye is rendered difficult because here, as a rule, there is also a more or less marked traumatic endophthalmitis in addition to the special form of inflammation. The clinician, then, directs his efforts rather toward recognition of those traumatized eyes which are liable to become the seat of sympathetic ophthalmia and, as such, should be enucleated. Such eyes are said to be sympathetically potent. Wounds in the so-called dangerous zone and those in which there are retained foreign bodies are particularly sympathetically potent. To avoid an occasional disastrous result through attempting too close a distinction between the sympathetically potent and the sympathetically nonpotent eye, the rule of the ophthalmologist in this regard is intentionally made broader than it perhaps need be. All eyes are regarded as sympathetically potent which have suffered perforating injuries which have not healed perfectly and especially those which have suffered from endophthalmitis or its results.

Enucleation may at times fail to prevent the development of sympathetic ophthalmia in the remaining eye even when that eye showed no evidence of inflammation at the time of enucleation. Microscopy with the slit lamp might perhaps explain some of these cases by demonstrating early changes not detectable by other methods of examination.

The following quotation from Butler<sup>29</sup> may serve to point out the importance of examinations with the slit lamp with regard to sympathetic ophthalmia :

It is impossible to overestimate the importance of a slit-lamp examination, of repeated examinations, in cases in which there is the slightest suspicion of this formidable complication. Often the diagnosis depends absolutely upon the result of these investigations, and they must never be omitted. The verdict of the slit-lamp compels us to remove eyes which appear harmless and enables us to retain those that are apparently dangerous.

The characteristic sign of a sympathetic inflammation, a sign which it shares with allied conditions such as tuberculous cyclitis, severe cyclitis in general, and with exudative choroiditis, is the presence of cells in the vitreous, the retro-lental space, and the anterior chamber; in a word, cells in the aqueous. Later signs are pellucid K.P. [*Keratitis Punctata*] and exudative nodules on the iris.

If in the case of an injured eye, which is under suspicion of being a possible exciting eye, we examine the aqueous and fail to find cells in the anterior chamber, in the retro-lental space, or in the vitreous, then it is very improbable that the eye is dangerous from the sympathetic standpoint. At the present time there is not sufficient evidence to make the statement more positive.

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29. Butler, T. H.: *An Illustrated Guide to the Slit-Lamp*, New York, Oxford University Press. 1927.

If, on the other hand, in addition to the ordinary clinical signs we find an infiltration of the vitreous with cells, with or without cells in the retro-lental space, and the anterior chamber, then the presumption that the eye is dangerous is very greatly strengthened.

Finally, if cells are found in the same situations in the fellow eye, with or without more obvious signs such as K.P., and ciliary injection, then the eye is sympathizing, and active treatment must be initiated at once, even if the signs of inflammation are slight.

Whenever the histologic picture of sympathetic ophthalmia is found in an enucleated eye, the danger of development of a sympathizing process may still exist for a number of weeks after enucleation.

In consideration of the clinical evidences of inflammation in a sympathizing eye, it is customary to differentiate between sympathetic irritation and sympathetic inflammation. While the former is sometimes the forerunner of the latter, it is not necessarily a part of sympathetic ophthalmia. It consists of such subjective symptoms as photophobia, lacrimation and, at times, pain or weakness of accommodation. Objective evidences of inflammation are lacking. Sympathetic inflammation, on the other hand, gives rise to the subjective and objective phenomena which are characteristic of sympathetic ophthalmia as occurring in the sympathizing eye. These phenomena may be summarized as those of a low grade progressive uveitis usually terminating in complete blindness and atrophy of the bulb, although more favorable outcomes are not uncommon.

The question of enucleation of an injured eye after a sympathizing process has broken out is discussed as follows by Salzmann<sup>30</sup> in Fuchs' "Diseases of the Eye," from which much of the foregoing clinical outline was abstracted:

It is conceivable that the sympathetic ophthalmia will course more mildly when the focus from which it has started is removed. On the other hand, the eye which excites the sympathy may retain some vision while the eye which suffers in sympathy may become entirely blinded. In such a case one robs the patient of the very last possibility of retaining vision by the enucleation of the injured eye. One should therefore not enucleate when the condition of the exciting eye is better than that of the sympathizing eye.

Enucleation rarely comes into consideration in connection with the sympathizing eye. Prophylaxis of course here falls by the way. Enucleation can only be justified by pain or disfigurement.

Aside from the prophylactic and possible palliative value of enucleation, the problems of therapy hardly fall within the scope of a paper of this kind. Mention might be made, however, of recent reports of

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30. Salzmann, M., in Fuchs, Ernst: *Diseases of the Eye*, ed. 15, translated by E. V. L. Brown, Philadelphia, J. B. Lippincott Company, 1933.

Woods and Little, Gill and others of very favorable results of the therapeutic use of uveal pigment—a phase of the problem of etiology to which reference will be made later.

#### ETIOLOGY AND PATHOGENESIS

As early as 1844, Mackenzie<sup>31</sup> had considered three pathways by which sympathetic inflammation might be transmitted, namely, through the vessels by means of their anastomoses within the skull, along the ciliary nerves or through the retina and optic nerves. Concerning these possible routes, he said, "The vessels on the side of the injured eye, being in a state of congestion which may increase to inflammation, perhaps communicate a disposition to similar disease to the vessels on the opposite side, with which they anastomose inside the cranial cavity." And again, "The ciliary nerves of the injured eye might be the paths along which the irritation is conveyed, through the medium of the third and fifth pairs, to the brain, from which it is reflected along corresponding nerves of the opposite side." Concerning the optic nerves, he wrote, "It is extremely probable that the retina of the injured eye is in a state of inflammation which advances along the corresponding optic nerve to the chiasma. From there, the irritative condition to which the inflammation was due crosses over to the retina of the opposite eye, along its corresponding optic nerve." Mauthner,<sup>32</sup> in 1878, said of Mackenzie that his observations "contain very nearly all that has been discovered in this province in the last forty years." Reviewing the development of the theories of pathogenesis, Mauthner leads up to a statement of his own conclusions from which a few passages may serve to illustrate the mystery in which the problem was then clothed.

We have, on the whole, no right at all to ask whether the sympathetic affection is transmitted along the optic nerves, or along the ciliary nerves. . . . For the transmission may be effected in both ways. But by this, however, we are not to understand that one and the same morbid process can be transmitted, now along the one path and now along the other. On the contrary, irritative and inflammatory conditions are transmitted from the optic nerve and retina, along the optic nerves; whilst those inflammatory processes which are chiefly observed in that portion of the eye which is nourished by the ciliary nerves, and especially in the uveal tract, are transmitted along the ciliary nerves.

In the present state of our knowledge we know nothing of any . . . direct transmission [by way of blood vessels].

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31. Mackenzie, W.: *Practical Treatise on the Diseases of the Eye*, London, Longman [and others], 1830; ed. 2, 1835; ed. 3, 1839, and ed. 4, 1854. The quotations are from Mauthner.<sup>32</sup>

32. Mauthner, L.: *Sympathetic Diseases of the Eye*, translated by Warren Webster and James A. Spalding, New York, William Wood & Company, 1881.

It is, of course, hard to say wherein the irritative condition consists; but it is a fact that the irritation can propagate itself to the second eye. . . .

Some one may ask how it is possible for such a connection to exist between the eyes, by means of the optic nerves, in those cases in which the optic nerve of the eye first affected is in a stage of total atrophy. A cord of connective tissue cannot transmit such a sensorial irritation. Granted; but even if this is so, we cannot assume . . . that *all* the fibers of the optic nerve of the first eye are atrophic.

By the beginning of the twentieth century bacterial theories had been advanced in explanation of the etiology, and there had been recorded many fruitless attempts to attach significance to the finding of one or another of a variety of organisms in cases of sympathetic ophthalmia. These theories of etiology had become incorporated in new theories of pathogenesis which by this time constituted a formidable list.

Schirmer<sup>1b</sup> (1900) classified the then current theories as follows:

1. Pure nerve theories
  - (a) Optic nerve theory
  - (b) Ciliary nerve theory
2. Pure bacterial theories
  - (a) Transmission by metastasis
  - (b) Transmission by reversed venous flow
  - (c) Transmission by lymph channels
3. Combined theories
  - (a) Meyer's theory
  - (b) Schmidt-Rimpler's theory

Many of these theories need mention today only because of their historical interest. The pure nerve theories were based on the conception of transmission of irritation after the manner of transmission of sensory or motor impulses. Müller's<sup>33</sup> objection to the optic nerve theory, based on the fact that the nerve frequently is completely atrophic, and Mauthner's attempt to overcome this objection will serve to illustrate this point of view.

The theory of bacterial transmission by metastasis, while foreshadowed by Mackenzie's suggestion of a blood vascular route, was first advanced by Berlin<sup>34</sup> (1880). Parsons,<sup>5</sup> in reviewing these theories, singled out this one as being the most probable. He referred to the fact that certain tissues furnish sites of predilection for certain organisms such as tetanus bacilli or typhoid bacilli and suggested that the organism causing sympathetic ophthalmia "is pathogenic only for the eye." He drew an analogy between this disease and others which set up metastases, such as syphilis, tuberculosis and leprosy in which

33. Müller, H.: Arch. f. Ophth. 4:363, 1858.

34. Berlin, R.: Samml. klin. Vortr. 186:1529, 1880.

the lesions are subacute or chronic, and not purulent, and contended that the fact that sympathetic ophthalmia "manifests itself as a uveitis both in the exciting and in the sympathizing eye points to the same *causa causans*." He concluded his discussion with the following hypothetic outline of events:

The organism multiplies in the exciting eye; if this is removed early infection [of the other eye] does not occur, but if organisms have escaped in quantity into the circulation sympathetic ophthalmia may follow. The conditions of existence in the circulation and in other organs of the body are relatively bad, as is shown in the case of other organisms. It is probable that early encapsulation may occur in the exciting eye. As is well known bacteria may long lie dormant but viable in this condition. Slight injury or other cause may set them free, thus explaining the occurrence of sympathetic ophthalmia long after the first injury, though preceded at a short interval by inflammation in the exciting eye.

The theory of transmission by reversed venous flow was based on the idea that bacteria might reach the cavernous sinus and then be carried into the veins of the opposite eye by a reverse flow of blood induced by coughing or sneezing. The theory was advanced by Arnold,<sup>35</sup> in 1891, but received little support.

The theory of bacterial transmission by lymph channels meant, according to most of its proponents, transmission by way of the inter-vaginal (subdural and subarachnoid) spaces of the optic nerves and their communications within the cranial cavity. Leber<sup>36</sup> (1881) originated the theory, and Deutschmann,<sup>37</sup> in supporting it, suggested the name ophthalmia migratoria for the disease. The evidence adduced in support of this theory was largely the result of animal experimentation in which pyogenic organisms were injected into the vitreous. The most convincing result was the production of a papillitis in the opposite eye, but even this was obtained by only a few of the many workers in this field. Römer,<sup>38</sup> in this connection, said that no conclusions concerning the pathogenesis of sympathetic ophthalmia in man can be drawn from experiments on animals with pyogenic organisms.

Parsons,<sup>5</sup> Ginsberg<sup>12</sup> and others pointed out that clinical evidence does not support this theory since the disease in the sympathizing eye relatively seldom begins with a papillitis. S. R. Gifford,<sup>39</sup> on the other hand, mentioned the frequent occurrence of optic neuritis in the second eye, "sometimes as practically the only symptom."

35. Arnold, J.: Virchows Arch. f. path. Anat. **124**:385, 1891.

36. Leber, T.: Arch. f. Ophth. **27**:272, 1881; **58**:325, 1904.

37. Deutschmann, R. H.: Ueber die Ophthalmia migratoria, Hamburg, Leopold Voss, 1889.

38. Römer, P.: Arch. f. Ophth. **55**:302, 1902.

39. Gifford, S. R.: Nebraska M. J. **14**:432, 1929.

E. Fuchs,<sup>40</sup> in 1905, opposed the theory on histologic grounds.

More recently, A. Fuchs<sup>41</sup> (1925) examined portions of optic nerve in seventy-five cases of sympathetic ophthalmia, and in almost one third of the cases found what he regarded as definite evidence of involvement of the nerve in the specific process. This consisted of lymphocytic infiltration of the pia extending from the tip of the intervaginal space backward for from 1 to 2 mm., although small, isolated foci could be found as far as 12 mm. back along the nerve. Reviewing this work, Ginsberg<sup>42</sup> concluded that proof of the migration of the process over the optic nerve cannot be discovered in Fuchs' findings.

Meyer's<sup>43</sup> theory stated that the results of ciliary irritation in the injured eye depended on the presence or absence of bacteria in the opposite eye. If bacteria were present, sympathetic inflammation resulted; whereas, if bacteria were absent, the result was only a sympathetic irritation.

Schmidt-Rimpler's<sup>42</sup> theory was based on the assumption that irritation of the ciliary nerves of one eye exerts a reflex influence on the circulation and nutrition of the other eye, thus paving the way for infection by some unknown agent. H. Gifford<sup>43</sup> contended that this theory would not stand the "test of clinical experience." He asked "If sympathetic ophthalmia depends merely on the coincidence of ciliary irritation and the accidental presence of germs in the circulation, why should we never hear of sympathetic ophthalmia in the tedious ciliary irritation existing with the numerous cases of one-sided keratitis, whether of trachomatous, herpes, phlyctenular, or other origin?"

The bacterial theories are all open to the important objection that micro-organisms have rarely been found in eyes which are affected by sympathetic ophthalmia, and to those which have been found it has been impossible to attach any etiologic significance.<sup>44</sup> The toxic theory, based chiefly on these negative findings with regard to actual infection, attempted to explain the lesions of sympathetic ophthalmia as being due to the effects of bacterial metabolic products (Gorecki,<sup>45</sup> Rosenmeyer,<sup>46</sup>

40. Fuchs, A.: *Ztschr. f. Augenh.* 56:275, 1925.

41. Meyer, E.: *Rev. gén. d'opht.* 9:481, 1890 (quoted by Schirmer<sup>1b</sup>).

42. Schmidt-Rimpler: *Arch. f. Ophth. (Abt. 1)* 38:199, 1892.

43. Gifford, H.: *J. A. M. A.* 34:341, 1900; *Ophth. Rec.* 25:344, 1916; also for review up to 1919, Wood, C. A.: *Encyclopedia of Ophthalmology*, Chicago, Cleveland Press, 1920, vol. 16, p. 12369.

44. The recent findings of J. Meller offer a striking exception to this statement.

45. Gorecki, in discussion on Trousseau: *Compt. rend. Soc. d'opht. de Paris*, April, 1891 (quoted by Schirmer<sup>1b</sup>).

46. Rosenmeyer, L.: *Arch. f. Augenh.* 28:71, 1893.

Bacchi,<sup>47</sup> Praun,<sup>48</sup> and others). Parson's objection that the disease in the sympathizing eye continues (or may even commence) after removal of the exciting eye is based on the assumption that the exciting eye furnishes the only focus from which such toxic substances may emanate. The idea of an extra-ocular focus of infection is advanced in one of the more modern theories which will be discussed later.

Since 1900 many of the theories in Schirmer's list have been left by the wayside, while others have survived and appear, more or less modified, among the group of so-called modern theories of etiology and pathogenesis to which more detailed consideration will be given.

The cytotoxic theory of Galowin<sup>49</sup> (1904) assumed that degenerating cells in the exciting eye give rise to an autocytoxin which is antigenically specific for uveal tissue. Galowin prepared a serum which, theoretically, contained this cytotoxin. Intra-ocular injection of the serum caused a severe iridocyclitis, while intravenous injection gave what Galowin believed were slight microscopic changes in the ciliary epithelium.

The mycotic theory advanced by Römer<sup>38</sup> (1902) and the so-called biologic theory proposed by H. Gifford<sup>39</sup> (1900) are variations of the older bacterial metastatic theory. The biologic theory assumes that germs surviving the struggle for existence in the first eye acquire an increased capacity for growing in uveal tissue. Transmission to the second eye occurs by way of the blood stream.

S. R. Gifford<sup>39</sup> pointed out the convenience of the biologic theory in explaining the improvement in the sympathizing eye which sometimes follows enucleation of the exciting eye. Removal of the first eye, he maintained, is comparable to elimination of a focus of infection. When improvement does not follow enucleation "it must be assumed that enough [germs] have already arrived so that the secondary focus is unable to destroy them." This, like the other theories of infection, has lacked the support of any conclusive evidence of infection in the lesions of sympathetic ophthalmia.

The more recent researches into the etiology and pathogenesis of sympathetic ophthalmia can be divided into three main groups which might be designated as follows: (1) investigations of the possible rôle of allergy either as the sole etiologic agency or as a factor in the production of the disease; (2) experiments based on the idea that the disease is caused by a filtrable virus; (3) efforts to explain the disease as a reaction to toxins (particularly tuberculous toxin) originating in some extra-ocular focus.

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47. Bacchi, A.: *Internat. Ophth. Cong.*, Rome 6:97, 1894.

48. Praun, E.: *Die Verletzungen des Auges*, Wiesbaden, J. F. Bergmann, 1899.

49. Galowin, S.: *Klin. Monatsbl. f. Augenh.* 7:150, 1909.

The so-called anaphylactic theory of pathogenesis was founded on the work of Elschnig<sup>50</sup> who, in 1910 and 1911, in a series of articles, reported the results of his investigations of the antigenic properties of uveal emulsions.

Elschnig's experiments with rabbits and guinea-pigs tended to show that animals can be sensitized to homologous or heterologous uveal emulsions; that the uvea is antigenically organ specific and not species specific, and that the constituent of the uvea responsible for its antigenic properties is the uveal pigment. Elschnig employed complement-fixation methods to demonstrate the development of specific antibodies in animals immunized to uveal tissue emulsions.

Woods,<sup>51</sup> since 1917, has extensively investigated this problem. He confirmed Elschnig's findings concerning the antigenic properties of uveal pigment and obtained additional evidence by using perfusion methods in pigment-sensitized dogs. Both Elschnig and Woods produced an apparently anaphylactic uveitis in animals, which resembled sympathetic ophthalmia in man. Both by animal experimentation and by clinical investigation of human material. Woods has produced evidence to show that, following injury to the uveal tract, the appearance of pigment antibodies (as demonstrated by complement fixation) can be interpreted as indicating a developing immunity to uveal pigment. He consistently was unable to demonstrate such antibodies in cases of sympathetic ophthalmia. In his later work, Woods substituted the intracutaneous test (with uveal pigment as antigen) for the less practical complement-fixation test and found hypersensitivity to uveal pigment, as evidenced by a positive intracutaneous test, in cases of sympathetic ophthalmia.

Unfortunately, it is not possible here to recount in greater detail these interesting experiments and clinical investigations which have furnished a basis for the idea that sympathetic ophthalmia is an anaphylactic, or allergic, phenomenon caused by absorption of uveal pigment.

Other investigators have obtained experimental results agreeing, wholly or in part, with findings of Elschnig and of Woods.

Fuchs and Meller,<sup>52</sup> in 1914, were unable to demonstrate uveal antibodies in serums of patients with sympathetic ophthalmia.

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50. Elschnig, A.: *Arch. f. Ophth.* **75**:459, 1910; **76**:509, 1910; **78**:549, 1911. Elschnig, A., and Salus, R.: *ibid.* **79**:428, 1911.

51. (a) Woods, A. C.: *Arch. Ophth.* **46**:503, 1917; *Tr. Sect. Ophth., A. M. A.*, 1917, p. 133; *Arch. Ophth.* **47**:161, 1918; *J. A. M. A.* **77**:1317, 1921; *Arch. Ophth.* **51**:451, 1922. (b) Woods, A. C., and Knapp, A.: *ibid.* **51**:560, 1922. (c) Woods, A. C.: *Tr. Ophth. Soc. U. Kingdom* **45**:208, 1925. (d) Woods, A. C., and Little, M. F.: *Arch. Ophth.* **9**:200, 1933.

52. Fuchs, A., and Meller, J.: *Arch. f. Ophth.* **88**:282, 1914.



Fodor,<sup>53</sup> in 1927, reported the results of a hundred examinations the results of which were somewhat in conflict with those of Woods. He agreed that antibodies are absent in sympathetic ophthalmia, but decided that it is unsafe to employ the results of cutaneous tests as a basis for enucleation.

Verhoeff<sup>54</sup> (1927) found the intracutaneous test negative in a case of sympathetic ophthalmia, contrary to Woods' experience.

Berens and others<sup>55</sup> in 1927 reported negative results of intracutaneous tests in eighteen cases "which," they wrote, "should be expected if Woods' findings are correct, as no case presented signs or symptoms of inflammation."

S. R. Gifford,<sup>56</sup> in 1929, stated "we have made tests on two cases of sympathetic ophthalmia and two of favorably reacting penetrating wounds, without obtaining any positive skin tests."

Gill<sup>56</sup> (1930) reported positive intracutaneous tests in sympathetic ophthalmia and negative tests in other diseases. He also noted that "clinical improvement, at times spectacular, follows the employment of uveal pigment therapy in sympathetic ophthalmia, provided it is used early in the disease. Enucleation is often avoided by using pigment therapy early in sympathetic ophthalmia."

A great many objections have been raised to the idea that sympathetic ophthalmia is caused by allergy toward uveal pigment. The antigenic properties of the uvea (considered as an organ) are not admitted as proved beyond doubt, and the concept that in sympathetic ophthalmia one becomes allergic to his own uveal pigment is considered untenable by many. One of the chief objections is expressed by S. R. Gifford<sup>56</sup> as follows: "On such a theory, sympathetic ophthalmia would be expected to occur fairly often following any iridocyclitis, whereas it occurs almost exclusively following a penetrating wound."

Another of the more interesting of the modern theories of etiology is the tuberculotoxic theory advanced by Guillery<sup>57</sup> in 1923. The marked similarity of the lesions of sympathetic ophthalmia to certain tuberculous lesions suggested to Guillery the possibility that tuberculous toxin might be the cause of this peculiar disease entity. This idea was also given support by the results of his experiments with various other bacterial toxins and other poisons in which exudative and pro-

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53. Fodor, G. I.: *Klin. Monatsbl. f. Augenh.* **79**:759, 1927.

54. Verhoeff, F. H.: *Arch. Ophth.* **56**:28, 1927.

55. Berens, C., and others: *Atlantic M. J.* **30**:547, 1927.

56. Gill, W. D.: *South. M. J.* **23**:885, 1930.

57. Guillery, H.: *Ztschr. f. Tuberc.* **38**:1, 1923; *Arch. f. Augenh.* **94**:143, 1924; *München. med. Wchnschr.* **72**:298, 1925; *Klin. Monatsbl. f. Augenh.* **76**:567, 1926; *Arch. f. Augenh.* **99**:63, 1928.

iterative ocular lesions had been produced in rabbits. In his earlier experiments Guillery showed that a reaction might be obtained in tissues remote from the source of the tuberculous toxins. He introduced semi-permeable capsules containing live tubercle bacilli into the peritoneal cavities of rabbits. Necrosis of the liver with proliferation of bile ducts and reticulum developed, while in the vicinity of the capsule there was the usual foreign body (tuberculoïd) reaction. By this method, however, Guillery was unable to produce disease of the eyes even when they were injured by various means.

Guillery then inserted his little capsules directly into the eyes of rabbits using the ciliary pouch method and later, direct introduction into the vitreous chamber. There developed, first in the inoculated eye and somewhat later in its fellow, lesions which Guillery believed to be identical with those of sympathetic ophthalmia. He therefore concluded that tuberculous toxin is the cause of the disease.

To duplicate more closely the conditions which he conceived to exist in the case of human sympathetic ophthalmia, Guillery attempted to supply tuberculous toxins from an extra-ocular focus in the presence of a traumatic lesion of one eye of a rabbit. A capsule containing tubercle bacilli was introduced into the orbit through a cannula, and a wound of the ciliary body of one eye was made with a lancet. The result was a uveitis of the injured eye followed by similar changes in the uninjured eye.

The experimental results of Guillery have been essentially confirmed by Kelen<sup>58</sup> (1928) and by Poos and Samerius<sup>59</sup> (1933), but these workers and others have questioned Guillery's conclusions. Von Selye<sup>60</sup> (1925) reported that a test of the Guillery method on twenty animals showed negative results in respect to the undisturbed eyes, while the inoculated eyes showed a wide range of reactions including (in five cases) typical nodular tuberculosis. Some of von Selye's capsules, tested in glycerin bouillon, showed permeability to tubercle bacilli. Marchesani<sup>61</sup> (1928) doubted that the capsules used by Guillery could be made entirely impermeable to tubercle bacilli. Guillery, on the other hand, discussed at length the question of sterility of his capsules and their impermeability to bacteria. The failure of tubercles to develop in the immediate vicinity of a capsule introduced into the vitreous was taken as evidence of its imperviousness to bacteria, since tubercles formed at the site of inoculation when the inoculum was not enclosed in a capsule.

58. Kelen A.: *Russk. j. othal.* 8:32, 1928.

59. Poos, E. and Samerius, F.: *Arch. f. Ophth.* 124:565, 1933.

60. von Selye, A.: *16<sup>e</sup> Klin. Monatsbl. f. Augenh.* 72:553, 1924; *Deutsche med. Wochenschr.* 50:1598, 1924.

61. Marchesani O.: *Klin. Monatsbl. f. Augenh.* 81:868, 1928.

A proper evaluation of Guillery's contribution must await further experimental investigation.

Because of repeated failure of investigators to demonstrate bacteria in the lesions of sympathetic ophthalmia, attempts have been made to explain the disease on the basis of a filtrable virus. Von Szily,<sup>62</sup> in 1924, using material from human corneal herpes, produced uveitis in rabbits, which, in some cases, was transmitted to the opposite eye. His procedure, briefly, was as follows: He first inoculated a rabbit's cornea by scarification and after from two to three days, when a characteristic keratitis had developed, took off the superficial layers of the cornea and used this material for subsequent inoculations. A small fragment of the material was then introduced into one eye of each of a number of rabbits. The site of inoculation was a so-called ciliary pouch made by separating the ciliary body from the sclera through a small corneal incision.

Uveitis of the inoculated eye regularly resulted. After about two weeks, when this reaction was beginning to subside, about 10 per cent of the animals showed iridocyclitis of the opposite eye—usually of a more severe form than that which had been observed in the inoculated eye.

Histologic sections of both eyes, in these cases, showed a picture resembling that of sympathetic ophthalmia. Sections of the papilla and optic nerve showed a similar infiltration, and there were also foci of infiltration in the chiasm and farther back along the optic tract. In addition, meningitis developed and, in 50 per cent of the cases, corneal herpes of the intact eye. Von Szily concluded, cautiously, that he had been able "through an invisible exciting cause to produce disease processes coinciding with human sympathetic ophthalmia in the essential points (injury of the ciliary region, transmission to the second eye of similar disease)."

Abe,<sup>62</sup> in 1926, and Velhagen,<sup>63</sup> in 1928, confirmed the findings of von Szily, while Marchesani,<sup>64</sup> repeating the experiments, obtained negative results.

In this country, in 1927, Gifford and Lucic<sup>65</sup> duplicated von Szily's results and further demonstrated the presence of virus in the second eye by inoculations from this eye to a ciliary pouch in another animal. The second animal, in turn, showed involvement of the second eye, and a further successful transfer to the cornea of a third rabbit showed transmission of the virus through three generations from the sympa-

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62. Abe, T.: *Arch. f. Ophth.* **117**:375, 1926.

63. Velhagen, K., Jr.: *Arch. f. Ophth.* **119**:325, 1928.

64. Marchesani, O.: *Arch. f. Augenh.* **97**:575, 1926.

65. Gifford, S. R., and Lucic, L. H.: *J. A. M. A.* **88**:465, 1927.

thizing eye. Gifford and Lucic's sections, like those of von Szily, indicated extension of the process by way of the nerves and chiasm to the other eye. This they confirmed by positive inoculations with material taken from various points along this route. They also showed that uveitis of both eyes could be produced by injection of the virus into the region of the chiasm. The authors concluded that "the possibility of a filtrable virus with neurotropic properties similar to those of the herpes virus being the cause of sympathetic ophthalmia is given some support by this work."

These experiments represent the closest approach that has been made to the experimental production of sympathetic ophthalmia, but there are serious objections to the virus theory which are yet to be overcome. While the migration of the virus infection is clearly shown to proceed by the optic nerve route in the case of herpetic virus lesions in rabbits, no such migration has been conclusively demonstrated in the case of human sympathetic ophthalmia. The associated meningitis likewise is not characteristic of the human disease. Neither von Szily nor Gifford and Lucic claimed that herpes virus is the cause of sympathetic ophthalmia, but their findings suggest, in the words of the latter workers, "the possibility that a virus, perhaps not dissimilar to the herpes virus, may be the cause."

Attempts to demonstrate the presence of a virus by inoculation of animals with material from human cases of sympathetic ophthalmia have, to say the least, furnished no support for the virus theory. Marchesani,<sup>66</sup> Undelt,<sup>67</sup> Gifford and Lucic,<sup>68</sup> Meesmann and Volmer,<sup>69</sup> and Lešer<sup>70</sup> all attempted such inoculation but with essentially negative results. The method usually employed was to introduce portions of diseased human eyes into ciliary pouches in a number of rabbits. Only rarely was any disturbance produced in the second eye of a rabbit so treated, and the value of even these meager results seemed slight on the basis of histologic examination.

Samuels<sup>24</sup> (1932), from a study of the anatomic findings in a large series of cases of sympathetic ophthalmia, presented evidence which, in his opinion, lends support to the theory that the disease is caused by a primary infection. He attached particular significance to the finding of two cases in which the typical infiltration was limited to the site of injury, furnishing examples of apparently primary lesions at the site of a wound and supporting the idea that in sympathetic ophthalmia a specific infectious agent enters at this point.

66. Marchesani, O.: *Klin. Monatsbl. f. Augenh.* **75**:218, 1925.

67. Undelt, J.: *Klin. Monatsbl. f. Augenh.* **76**:825, 1926.

68. Gifford, S. R., and Lucic, L. H.: *Arch. Ophth.* **1**:468, 1929.

69. Meesmann, A., and Volmer, W.: *Arch. f. Augenh.* **98**:271, 1927.

70. Lešer, O.: *Časop. lék. česk.* **67**:934, 1928.

This opinion is in accord with the earlier conclusion of Marchesani<sup>71</sup> who, on similar grounds, considered that the lesion in the exciting eye was due to a noxa entering at the site of injury.

Woods and Little,<sup>51d</sup> in a recent report, discussed the development of "a new school of thought as to the pathology of sympathetic ophthalmia." Observations of Key,<sup>72</sup> Marchesani,<sup>73</sup> Riehni,<sup>74</sup> and others have given more and more substantial form to the idea, suggested by Woods in 1925, that sensitization of the uveal tissue (of both eyes) renders it susceptible to the action of other noxious agents. Woods and Little offered further evidence in support of this explanation of sympathetic ophthalmia by recording and analyzing the results of intracutaneous pigment tests in one hundred and fifty-three patients.

In this series positive pigment reactions occurred only in cases of penetrating wounds, either operative or traumatic. Among twenty patients with sympathetic ophthalmia, there were eighteen who gave positive reactions to pigment tests.

Five patients were grouped as having delayed, noninfectious, post-operative uveitis, apparently a new clinical entity. All of these patients showed hypersensitivity to uveal pigment as shown by intracutaneous tests, but in none of this group did sympathetic ophthalmia develop. Two patients of this group were given courses of pigment therapy which were attended by remarkable clinical improvement and loss of sensitization as shown by negative intracutaneous tests. Among the group of patients with sympathetic ophthalmia those treated with uveal pigment, according to the authors, did vastly better than those not treated with pigment.

In the light of these and other observations, the authors conclude that "in sympathetic ophthalmia hypersensitivity to uveal pigment is a general rule, although patients with acute exacerbations of the disease may have a definite phase in which the intracutaneous test is negative." The occurrence of cases of chronic uveitis in which hypersensitivity to pigment developed without sympathetic ophthalmia supported the contention that "pigment hypersensitivity does not appear to be the cause per se" but that "some other factor enters into the disease."

An interesting feature of Woods and Little's report is an account of the histologic study of specimens of skin taken from the site of intracutaneous pigment tests. The study was made at the suggestion and with the collaboration of Jonas S. Friedenwald. Skin from two normal persons, who had been given intradermal injections of pigment

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71. Marchesani, O.: *Ztschr. f. Augenh.* **56**:275, 1925.

72. Key, B. W.: *Am. J. Ophth.* **9**:574, 1926.

73. Marchesani, O.: *Zentralbl. f. d. ges. Ophth.* **22**:804, 1930.

74. Riehni, W.: *Deutsche med. Wchnschr.* **55**:907, 1929; *Arch. f. Ophth.* **123**:361, 1930; *Arch. f. Augenh.* **105**:82, 1931.

two weeks before, showed "a moderate infiltration with monocytes and lymphocytes around the injected pigment. The larger part of the pigment remained free in the tissues not ingested by phagocytic cells." Sections of skin from two persons giving positive intracutaneous tests showed the deposits of pigment "infiltrated with great numbers of epithelioid cells and giant cells in which the granules were phagocytosed." Both of the latter specimens were from persons cured of sympathetic ophthalmia, the excisions being made two and four weeks, respectively, after the injection of pigment.

Friedenwald,<sup>26</sup> in discussion of these results, emphasized the resemblance of the positive cutaneous lesions to the uveal lesion in sympathetic ophthalmia.

Within the past year, Meller<sup>75</sup> reported bacteriologic observations which are of interest. In the laboratory of E. Löwenstein, by a special culture method it is reported, virulent tubercle bacilli have been recovered first, from the blood stream in a case of sympathetic ophthalmia, then from the ocular tissue in a case of spontaneous iridocyclitis and, lastly, from the circulating blood and the tissues of the exciting eye in a typical case of sympathetic ophthalmia.

The last-mentioned case was one of a perforating injury in which a sympathizing inflammation was noted six weeks after the injury. On the day of the outbreak of disease in the second eye a blood culture was made, the injured eye was enucleated, and tissue from its interior was cultured.

The histologic changes in the enucleated eye were reported in detail by Meller, who epitomized them as showing "das volle Bild der sympathisierenden Uveitis in allen seinen Einzelheiten" (the complete picture of sympathetic uveitis in all its details).

The cultures of ocular tissue showed a luxuriant growth of tubercle bacilli, while the cultures of the blood, although also positive, showed only a sparse growth. Blood taken ten days after enucleation showed the bacteremia still present. The virulence of the organisms was proved by inoculation into animals.

Meller expressed the belief that sympathetic ophthalmia is a bacterial disease caused by the tubercle bacillus. He pointed out that particularly people who have a bacteremia at the time of injury to the eye are in danger of developing a tuberculous uveitis. When the tuberculosis is of a proliferative character it can be called sympathetic ophthalmia, and the other eye is likely to become involved in the process. According to Meller, uveitis does not develop in all of those cases of injury in which there is a coexisting bacteremia, and, on the other hand, bacteremia

75. Meller, J.: *Wien. klin. Wchnschr.* 45:33, 1932; *Ztschr. f. Augenh.* 77:1, 79:75, 1932.

occasionally causes a uveitis in an untraumatized eye, but only when the bacteria are detained in the walls of the vessels. Hemorrhage into the tissues produces particularly favorable conditions for invasion and incubation of the bacteria brought by the circulating blood. The importance of wounds of the ciliary body is explained on this basis. The author admits the possibility of exogenous tuberculous infection at the site of injury but believes that the infection is usually hematogenous.

Meller emphasizes the vascular phenomena in various portions of the eye as being of particular importance, since the proliferation of cells into the vessels favors spread of the infection by way of the blood stream. The involvement of the second eye is regarded as a metastatic phenomenon caused by blood-borne virulent tubercle bacilli from the first eye. Many of the peculiarities of the disease can be explained, according to Meller, by this concept.

Interesting and suggestive as are the findings of Meller, they can hardly justify the conclusion that the enigma of sympathetic ophthalmia has at last been solved. Meller's work must first be confirmed. It must further be shown that the bacilli can regularly be recovered from the ocular tissues in these cases, and the cultural method, or methods, employed must be of proved reliability.

#### CONCLUSIONS

Any attempt to summarize and draw conclusions concerning the various disputed aspects of sympathetic ophthalmia would serve only to add opinions of doubtful value to an already long and conflicting list. The reader, I believe, might better be left to form his own conclusions or to await further elucidation of the many interesting problems which the disease presents.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Knighthood has been conferred on Robert Muir, professor of pathology in the University of Glasgow.

Roy Lee Moodie, anatomist and paleopathologist, has died at the age of 54. He made important contributions to the study of the structural evidences of ancient disease.

Michael Heidelberger, associate professor of biological chemistry at Columbia University, has been awarded a Guggenheim fellowship for research at the University of Uppsala, Sweden, on the molecular weight of thyroglobulin.

Richard Kockel, professor of forensic medicine in the University of Leipzig, has died at the age of 69.

Ernest Scott, professor of pathology in the Ohio State University since 1915, has died of coronary thrombosis, at the age of 58.

**Society News.**—At the meeting of the American Association of Pathologists and Bacteriologists in Toronto, March 29 and 30 last, William Boyd was elected president, N. C. Foote vice president, H. T. Karsner secretary and F. B. Mallory treasurer. The next meeting will be held at the Cornell University Medical College, New York, on April 18 and 19, 1935.

The annual meeting of the American Society for the Study of Neoplastic Diseases (president, Max Cutler) will take place in Washington, D. C., on September 6 to 8, 1934.

The William Wood Gerhard Gold Medal of the Pathological Society of Philadelphia was presented to George H. Whipple of the University of Rochester School of Medicine and Dentistry at a meeting on April 12, 1934. At that time Dr. Whipple gave the Annual Conversational Lecture of the society on "Regeneration of Hemoglobin and Blood Plasma Proteins Controlled by Diet Factors."

**Medicolegal News.**—The University of Michigan has organized an Institute for Law Enforcement Officers. Recently four all-day sessions, attended by more than a hundred officials, were held at Ann Arbor with lectures and discussion on poisoning, the examination of dead bodies, wounds, personal identification and other topics of criminologic interest.

**Gibbs Prize to Be Awarded.**—The New York Academy of Medicine announces that a sum of about \$800 is available under the Edward M. Gibbs Memorial Prize toward original research in diseases of the kidney in 1934. Candidates for the award must be physicians who have been graduated at least three years and who are residents of the United States. They must submit evidence of research already performed and facilities to prosecute research on the causation, pathology and new methods of treatment of diseases of the kidney. The award may be continued through not more than three years to any one person. Applications with the required evidence should be sent to the New York Academy of Medicine, 2 East One Hundred and Third Street, New York, before June 1, 1934.

**Historic Microscopic Sections.**—The nephritis clinic at Guy's Hospital, London, has presented to the department of physiology of the University of Cincinnati microscopic sections of three kidneys described by Richard Bright in his "Reports of Medical Cases," 1827, and in *Guy's Hospital Reports*, 1838.



# Abstracts from Current Literature

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## Pathologic Anatomy

RUPTURE OF THE AORTA. F. C. NARR and A. H. WELLS, *Am. Heart J.* **8**:834, 1933.

An interesting case is described of partial rupture of the aorta a little above the attachment of the aortic valve, due to so-called idiopathic medial necrosis, followed by dissecting aneurysm, with subsequent rupture of the outer coat into the pericardium. This rupture was rapidly fatal. The patient was a physician, 29 years old.

R. J. NIEHAUS.

THE INCIDENCE AND SEVERITY OF ARTERIOSCLEROSIS IN THE ORGANS FROM 500 AUTOPSIES. WILLIAM B. WARTMAN, *Am. J. M. Sc.* **186**:27, 1933.

An analysis of the incidence and severity of arteriosclerosis in the organs in 500 unselected autopsies is presented. The order of frequency with which the arterioles and small arteries of the organs were affected was: spleen, brain, kidney, suprarenal gland, pancreas, heart, gastro-intestinal tract, lungs, liver and diaphragm. This is in agreement with the observations of most investigators. The arteries of the organs which were most frequently involved were also most markedly affected; conversely, those of organs least often involved showed mild degrees of arteriosclerosis. The vessels of the diaphragm were frequently implicated. The incidence and degree of arteriosclerosis increased with the age. The reports from the literature and the results of this study indicate that a generalized arteriosclerotic process of a degree sufficiently advanced to be of clinical importance occurs infrequently.

AUTHOR'S SUMMARY.

RENAL LESIONS ASSOCIATED WITH MULTIPLE MYELOMA. E. T. BELL, *Am. J. Path.* **9**:393, 1933.

Renal insufficiency develops frequently in multiple myeloma, especially in the advanced stages. In some instances it is due to arteriosclerosis; in others it is caused by pyelonephritis resulting from compression of the spinal cord or hypertrophy of the prostate. The only direct effect of multiple myeloma on the kidneys is that due to the formation of tubular casts of Bence-Jones protein that obstruct the tubules and cause atrophy. When large numbers of tubules are obstructed, extensive atrophy of the cortex and renal insufficiency ensue. There is no evidence that the Bence-Jones protein injures either the tubules or the glomeruli. Cortical atrophy is caused by obstruction and should not be interpreted as "nephrotic contracted kidney." In one instance renal insufficiency was apparently caused by the accumulation of a highly concentrated protein solution in the glomerular capillaries. In two other instances the same condition was found in a few capillaries.

AUTHOR'S SUMMARY.

EXTREME ALTERATION OF THE AORTIC VALVE IN SYPHILITIC AORTITIS. O. SAPHIR and J. STASNEY, *Am. J. Path.* **9**:431, 1933.

Two cases of syphilitic aortitis with involvement of the aortic valve are reported. In both instances the involvement of the valve reached extreme degrees, causing transformation of one sinus of Valsalva into a cavity in one instance and almost complete disappearance of a part of one sinus of Valsalva in the other. Reports of only eight similar cases were found in the literature. Histologically, granulation tissue and much fibrosis and hyalinization were found within the commissures and

the adjacent parts of the adherent aortic cusps. The granulation tissue had spread from the aortic intima through the commissures and also through the base of the cusps into the cusps themselves. The widening of the commissures, which is pathognomonic of syphilitic involvement of the aortic valve, was the result of adhesions between the cusps of the aorta and the corresponding aortic wall. The widening of the commissures, therefore, cannot be explained on a mechanical, but must be explained on an inflammatory, basis.

AUTHORS' SUMMARY.

RHEUMATIC HEART DISEASE WITHOUT VALVULITIS. C. Z. GARBER, *Am. J. Path.* 9:443, 1933.

Seventeen cases have been noted in the literature in which the diagnosis of rheumatic fever was made at autopsy and in which all the valves of the heart were apparently free from rheumatic lesions; all these cases exhibited myocarditis with Aschoff bodies present, and in four cases pericarditis was also present. An additional case of chronic rheumatic fever is reported, based on present criteria, in which the valves of the heart were free from rheumatic lesions. Aschoff bodies were not found, and the diagnosis of rheumatic fever depended on the presence of characteristic lesions in the left auricle, scars in the media and diffuse infiltrations of the heart and of the adventitia of the aorta and the pulmonary artery by inflammatory cells. Death occurred one year and eight months after the first symptoms of congestive heart failure, following administration of quinidine. An embolus, accounting for the mode of death, was found in the right internal carotid artery. It is believed that this was formed on the rheumatic endocardial lesion in the left auricle.

AUTHOR'S SUMMARY.

THE RELATION OF HEPATITIS TO CHOLECYSTITIS. J. F. NOBLE, *Am. J. Path.* 9:473, 1933.

Infiltration of the portal spaces by lymphocytes and polymorphonuclear leukocytes is seen almost constantly in the liver at autopsy. This portal infiltration seems to have no particular relation to generalized infections or to any specific type of disease and apparently bears no relation to the nonclinical cholecystitis frequently found in routine postmortem examinations. The latter is seen less frequently in the female than is clinical cholecystitis and is more frequent in the younger age groups. In the series of autopsies studied cirrhosis occurred too rarely to justify the conclusion that the hepatitis described has an etiologic relation to cirrhosis.

AUTHOR'S SUMMARY.

BONE MARROW IN TULARAEMIA. R. D. LILLIE and E. FRANCIS, *Pub. Health Rep.* 48:1127, 1933.

Focal lesions were almost constantly present in the bone marrow of rodents with acute tularemia in the five species of rodents in which the marrow was systematically studied. They were frequent in the bone marrow also of rabbits and guinea-pigs with subacute tularemia. The focal lesions in the marrow in subacute tularemia often became granulomatous, but also often remained as simple focal necrotic changes, while lesions in other organs were granulomatous. There was a greater tendency to granulomatous reaction in rabbits which had been repeatedly inoculated with living cultures of *Bacterium tularensis*; but in some of these in which marked granulomatous reactions were present in the lungs within a few days after the last inoculation, the lesions were in all probability assignable to the inoculation carried out a month or more previously. Aside from focal lesions, there appeared to be some destructive action affecting the more mature cell forms of the marrow. It appears probable that focal lesions may be encountered in the bone marrow of man when a more extensive search is made.

A CASE OF GONOCOCCAL ENDOCARDITIS WITH RUPTURE OF THE AORTIC VALVE AND DEATH. WILLIAM B. PORTER, *Heart* **16**:201, 1933.

A case of gonococcic endocarditis is reported in which rupture of the aortic cusp was heard to take place during life. The rupture was followed immediately by acute and fatal edema of the lungs. A Negress, aged 16, entered the hospital with acute gonococcic vaginitis of four weeks' duration and acute arthritis of two days' duration. The heart showed no demonstrable enlargement. A soft systolic murmur was heard over the precordium. The pulse rate was 118 and regular. The blood pressure was 120 systolic and 65 diastolic. Three days after admission acute gonococcic conjunctivitis developed in the left eye. Nine days after admission the systolic murmur had definitely increased over the base of the heart, and a low-pitched diastolic murmur was audible along the left border of the sternum. A blood culture showed a growth of gonococci. Seventeen days after admission, while the heart was being auscultated, the diastolic murmur suddenly increased in intensity and became musical. The patient soon became dyspneic. Moist râles were heard over both lungs, and pink, frothy fluid began to flow from the nose and mouth. The pulmonary edema progressed rapidly. The pulse rate rose to 140. The patient became unconscious and died about twenty minutes after the onset of the pulmonary symptoms. Autopsy, performed the following day, revealed edema and congestion of the lungs. The right lung weighed 590 Gm., and the left, 600 Gm. There was a thin, blood-tinged fluid in the bronchi and the myocardium was soft and pale. The heart weighed 310 Gm. There was perforation of the two anterior aortic cusps, one perforation being 6 mm. and the other 4 mm. in diameter. A low verrucous, friable, pinkish-white vegetation was seen on the margins of the perforations. Microscopically, there were: edema, fibrinous exudation, leukocytic infiltration and moderate histiocytic proliferation in the cusps of the aortic valve, a small abscess in one cusp, scattered foci of interstitial polymorphonuclear infiltration in the myocardium, pulmonary alveoli filled with serous fluid in which were alveolar phagocytes containing blood pigment, and pulmonary congestion. Porter concludes that the change in the diastolic murmur was due to rupture of the aortic cusps, resulting in aortic regurgitation, cardiac failure and pulmonary edema.

ESTHER MARTING.

RENAL LESIONS IN ECLAMPSIA AND NEPHRITIS OF PREGNANCY. D. BAIRD and J. S. DUNN, *J. Path. & Bact.* **37**:291, 1933.

The common lesion in the kidneys in fatal eclampsia is glomerular and is characterized by thickening of capillary walls and of endothelium, leading to some degree of obstruction to the flow of blood. Tubular changes are less constant, and may be of secondary import. A degree of this lesion probably constitutes the anatomic basis of the albuminuria of pregnancy. In a case of renal cortical necrosis diffuse glomerulitis had existed prior to the occurrence of the necrosis.

AUTHORS' SUMMARY.

INTERPRETATION OF RENAL CHANGES IN LIPOID NEPHROSIS. P. GÉRARD and R. CORDIER, *Arch. internat. de méd. expér.* **8**:225, 1933.

Gérard and Cordier injected normal serum and serum rich in cholesterol intraperitoneally in a series of salamanders and frogs under varying conditions. Then the kidneys were fixed in solution of formaldehyde, and frozen sections were made and examined with Nicol prisms under polarized light. They found that cholesterol cannot filter through normal glomerular epithelium. However, if this is rendered permeable by previous injection of egg albumin, cholesterol comes through and is deposited in the epithelium of the convoluted tubules. In clinical lipoid nephrosis the albuminuria renders the glomerular epithelium permeable to cholesterol, which thus enters the convoluted tubules and is there reabsorbed by the epithelium.

JACOB KLEIN.

**CALCIFICATION OF THE CARDIAC SKELETON.** WILLY GIESE, *Beitr. z. path. Anat. u. z. allg. Path.* **89:16**, 1932.

According to Tandler, the skeleton of the heart consists of the fibrous framework which connects the musculature of the ventricles and the atria. In more than 700 autopsies, calcification of this portion of the heart was observed 72 times. There were 30 cases of calcification of the annuli fibrosi, 2 of calcification of the aortic valves and 22 of combinations of both. In 8 cases the calcification followed inflammation. Calcification did not occur as a rule before the fiftieth year of life and was more extensive after the sixth decade. The calcification was preceded by fatty degeneration, atheroma, focal necrosis and hyalinization of the collagenic fibers. The calcification often caused mitral or aortic vicia, and in a few cases calcification of the pars membranacea septi produced heart block. Senility, mechanical injuries and disturbance of the calcium metabolism may be the etiologic factors in this condition.

C. ALEXANDER HELLWIG.

**LIPOID SUBSTANCES IN CELLS OF MESENCHYMAL ORIGIN.** J. GOLDMANN, *Virchows Arch. f. path. Anat.* **287:587**, 1933.

Goldmann believes that two forms of lipid are to be found in the normal mesenchymal cell. One form, called by him "integral" lipid, is an integral part of the cell and is always present. The other form is the "infiltrative" lipid, the presence of which is dependent on the intake of lipoids.

A number of rabbits, guinea-pigs, rats and mice were fed a 5 per cent solution of cholesterol in oil for a period of from five to fifteen days. The animals were then killed, and various organs and blood smears were examined. Goldmann used his own modification for staining lipid with sudan and naphthol-hematoxylin. With this method it was possible to demonstrate the normal "integral" lipid within the leukocytes of the blood, as well as in cells of the various tissues. The intracellular lipid structure seems to be largely dependent on the protein structure; in pathologic conditions the lipid granules become separated from the protein and may disappear entirely. The reticulo-endothelial cells were found to be filled with "infiltrative" lipid throughout, without apparent damage of the cellular structure.

W. SAPHIR.

**THE PANCREATIC ISLETS OF THE NEW-BORN INFANT OF A DIABETIC MOTHER.** K. A. HEIBERG, *Virchows Arch. f. path. Anat.* **287:629**, 1933.

A woman with severe diabetes mellitus passed through pregnancy and delivery without any untoward events. The diabetic condition improved after delivery. The infant died two days after birth with symptoms of hypoglycemia; the blood sugar was 60 mg. per hundred cubic centimeters. Microscopic examination of the pancreas revealed marked hypertrophy and hyperplasia of the islets.

W. SAPHIR.

**KÖHLER'S DISEASE OF THE TARSAL NAVICULAR BONE IN IDENTICAL TWINS.** P. ESAU, *Virchows Arch. f. path. Anat.* **287:634**, 1933.

Köhler's disease, or os naviculare pedis retardatum, consists in a malformation of unknown cause. Although the disease may occur at times without any clinical symptoms, it is usually recognized fluoroscopically by the multitude of osseous nuclei and by the disturbances in calcification of the navicular bone. Esau presents the history and roentgenograms of a typical case of this disease in two single ovum twins. He concludes that Köhler's disease is due to a disturbance of embryonic development at an early stage of that development.

W. SAPHIR.

REGRESSION OF THE SACRAL INTERVERTEBRAL DISKS AND ITS POSSIBLE RELATION TO SACRAL CHORDOMA. R. SCHWALBE, *Virchows Arch. f. path. Anat.* **287**:651, 1933.

In this comprehensive and well illustrated article Schwalbe undertakes to follow the fate of the human sacral intervertebral disks. It is known that these disks are fully developed in the early stage of life, and that they undergo complete regression before osseous union of the five sacral vertebrae takes place.

Careful and thorough histologic examination was made of sixty-six sacral bones of persons of various ages. The youngest person whose sacral bones were examined was a premature infant 33 cm. long, while the oldest was a woman aged 84.

The phases of regression are histologically recorded and described. Osseous union of the sacral vertebrae takes place at the end of the second decade. But even in the later stages of life evidence of continued osseous and cartilaginous activity was frequently observed.

Rudimentary intervertebral segments of the chorda were found up to the fifth decade. The possible development of chordoma from cells of these rudimentary rests is discussed.

W. SAPHIR.

THE POROTIC FORM OF PAGET'S DISEASE OF THE SKULL. B. PINES, *Virchows Arch. f. path. Anat.* **287**:714, 1933.

A thorough study of a typical case of Paget's disease reveals that a porotic form exists, that is confined chiefly to the skull. Multilocular areas of porosis and markedly increased periosteal appositional growth of bone characterize this rare form of Paget's osteitis deformans.

W. SAPHIR.

CYST OF THE ROOF OF THE THIRD CEREBRAL VENTRICLE. M. A. BASS, *Virchows Arch. f. path. Anat.* **287**:790, 1933.

Cysts of the third cerebral ventricle originate either from the ependyma or from the choroid plexus. Only four ependymal cysts and two cysts of the choroid plexus were found described in the literature. Bass adds a further case of cyst of the choroid plexus. A woman, aged 22, died with symptoms of cerebral tumor. Autopsy revealed a cherry-sized cyst on the roof of the third ventricle, which had led to obstruction of the circulation of fluid, with subsequent hydrocephalus. The cyst was lined with cylindric plexus epithelium and contained a network of collagenic and reticulum fibers, in which endothelial cells, fibroblasts and numerous corpora arenacea were embedded.

W. SAPHIR.

PARTIAL CONGENITAL ABSENCE OF THE PANCREAS. CHRISTLIEB, *Virchows Arch. f. path. Anat.* **289**:241, 1933.

The author adds one to the small number of reported cases of congenital partial absence of the pancreas. The patient was a woman who died at the age of 69 of cerebral hemorrhage. On the day before death the urine contained a trace of sugar. Sugar had not been present when she was under treatment for pneumonia a year and a half before death. The pancreas measured 8 by 4 by 1.5 cm. and was closely attached in the concavity of the duodenum. It consisted of a small head, an uncinate process and a small part of the body. Histologically, the islets were numerous and large, a condition interpreted as compensatory. The anomaly is ascribed to failure of development of the dorsal anlage of the pancreas.

O. T. SCHULTZ.

CONGENITAL ATRESIA OF THE LARYNX. E. FRITZ, *Virchows Arch. f. path. Anat.* **289**:264, 1933.

The anomaly described occurred in a new-born male infant 43 cm. in length. Heart tones were heard for from twelve to fifteen minutes after birth; the application of cold water led to a single spasmodic attempt at respiration. The larynx

appeared normal externally, and the epiglottis was normal. The superior opening of the larynx was normal, but at a distance of 0.5 cm. below the opening the larynx was closed. The trachea was absent for a distance of 3 cm. below the larynx. The bronchi and a portion of the trachea 1.5 cm. long, superior to the bifurcation, were normally formed. A narrow fistula connected the esophagus with the lower portion of the trachea. The generalized edema and hypertrophy of the lungs that previous observers have noted in atresia of the larynx were apparently not present in this instance.

O. T. SCHULTZ.

ANATOMIC STUDY OF THE ESOPHAGEAL HIATUS OF THE DIAPHRAGM. R. NEUMANN, *Virchows Arch. f. path. Anat.* **289**:270, 1933.

This is an anatomic study of the esophagus and stomach in the region of the diaphragmatic hiatus through which the esophagus passes, undertaken because of the clinical importance recently attached to so-called insufficiency and hernia of the hiatus. In 250 unselected necropsies the region of the hiatus was studied by a technic devised to permit examination without injury to any of the attachments of the diaphragm. In 100 necropsies anatomic dissections of the region were made. The mechanics of the region was studied by traction of the esophagus and by filling the esophagus and stomach. The hiatus is bounded by muscular and musculotendinous tissue and is filled in by finely fibrillated elastic connective tissue that is covered superiorly by the pericardium and pleura and inferiorly by the peritoneum. The cardiac antrum is bounded cephalad by the hiatic sulcus and caudad by the cardiac or Arnold's sulcus. The cardiac antrum is neither anatomically nor physiologically a part of either the esophagus or the stomach. The gastric mucosa ceased at Arnold's sulcus in 53 per cent of the bodies examined; in 4 per cent it extended upward to the hiatic sulcus. Traction and filling revealed the presence of bulbous enlargements, termed "bulbi oesophagi" if situated superior to the hiatic sulcus and "bulbi antri cardiaci" if situated inferior to this sulcus. The bulbous enlargements varied in size from that of a walnut to that of an egg. They were present in 12.5 per cent of the bodies examined. No correlation was noted between the clinical or constitutional state and the presence of bulbous enlargements, except that every person with a short, thick habitus associated with emphysema and cachexia had a bulbous cardiac antrum. The bulbous enlargements were not true hernias. They were not associated clinically with the gastroduodenal symptom complex of Roemheld or the epiphrenic syndrome of von Bergmann. Their formation is physiologic and is due to changes in the elasticity of the tissue of the hiatus, increase in the negative intrapleural pressure and increase in the size of the hiatus.

O. T. SCHULTZ.

ACQUIRED DIAPHRAGMATIC HERNIA OF THE STOMACH. G. JUNGFER, *Virchows Arch. f. path. Anat.* **289**:463, 1933.

Jungfer describes a hernia of the stomach through an enlarged esophageal foramen into the left pleural cavity. The esophagus was short. The course of the blood vessels led him to conclude that the hernia was not congenital but had developed after birth.

O. T. SCHULTZ.

LYMPHOGRANULOMATOSIS OF THE UTERUS. M. UDDSTRÖMER, *Virchows Arch. f. path. Anat.* **289**:486, 1933.

Uddströmer collected all the instances of lymphogranulomatosis reported in Sweden between 1915 and 1931 in which the diagnosis was confirmed by histologic examination. Involvement of the pelvic organs by direct continuity from pelvic lymph nodes is not unusual. His material included a case, which he describes, in which the involvement of the uterus was diffuse, did not extend beyond the limits of the organ and was apparently of hematogenous origin.

O. T. SCHULTZ.

ARTERIAL DISEASE IN TYPHOID FEVER. Z. A. GASTEWA, *Virchows Arch. f. path. Anat.* **289**:636, 1933.

Gastewa presents two cases of typhoid fever, in one of which there was gangrene of the right foot, and in the other gangrene of both lower extremities and one upper extremity and terminal hemiplegia. Both patients were young persons, aged 15 and 21 years, respectively; hence senile vascular changes are to be excluded. The gangrene developed at the height of the disease, on the thirty-ninth day in the first case, during a relapse, and on the fourteenth day in the second case. Gangrene and hemiplegia were due to arterial thrombosis. In severe cases of typhoid fever the large and medium-sized arteries present localized areas of degeneration of the elastic tissue and necrosis of the connective tissue of the intima. The intimal changes may lead to thrombosis, which is the cause of the gangrene that sometimes complicates typhoid fever. The degenerative lesions of the arterial wall may be associated with tears into the media and through the wall.

O. T. SCHULTZ.

PERIOSTEAL BONE FORMATION AT THE EPIPHYSEAL LINE IN A CASE OF CONGENITAL SYPHILIS. G. GERSTEL, *Virchows Arch. f. path. Anat.* **289**:516, 1933.

Roentgenologic and anatomic examination of the lower end of each femur of a child who died at the age of 7 weeks of congenital syphilis revealed the presence of osseous outgrowths at the epiphyseal line. The bony masses had been formed by and within the thickened periosteum. The unusual lesion is interpreted as a regenerative reactive process evoked by the destructive osteochondritis that was present at the junction of the diaphysis and epiphysis. Spirochetes could not be demonstrated in the tissues; the patient had been under antisyphilitic treatment before death.

O. T. SCHULTZ.

HISTOLOGIC CHANGES IN THE CLINICALLY UNALTERED SKIN IN SYPHILIS. HELENE HERZENBERG, E. BANJAMOWITSCH and A. LEWIN, *Virchows Arch. f. path. Anat.* **289**:688, 1933.

Other observers have noted previously cellular proliferative reactions about the small blood vessels of the apparently normal skin of persons with syphilis. Although most of these observers considered the reaction nonspecific, Lukomski, in 1929, held it to be inflammatory and specific. Stadler accepted this view and attached great etiologic importance to the reaction in cardiovascular syphilis. Herzenberg, Banjamowitsch and Lewin examined histologically the macroscopically unaltered skin of thirty-four patients with syphilis in various stages. They observed a histiocytic proliferative reaction about the small vessels. But they also observed an identical reaction in the skin of a group of patients with diseases of the genito-urinary tract and of patients who had undergone operations and in the skin removed at necropsy from thirty subjects without syphilis. They interpret the reaction as hyperplasia of the perivascular connective tissue brought about by metabolic changes in the skin. It is not inflammatory in origin and has nothing to do with syphilis.

O. T. SCHULTZ.

THE PATHOGENESIS OF EPIPLOITIS. P. E. A. NYLANDER, *Arb. a. d. path. Inst. d. Univ. Helsingfors* **7**:1, 1933.

Two cases of omental inflammation are reported. In one a splinter of wood had perforated the gastro-intestinal tract and provoked a chronic inflammation of the omentum, with numerous giant cells. In the second case there was a chronic inflammatory swelling of the omentum, which was adherent to the transverse colon, probably as a result of a previous laparotomy. A variety of factors may cause omental inflammation, among which are previous operations, foreign bodies (fish bones, needles) and gastro-intestinal parasites, such as *Taenia*, *Oxyuris* and *Distoma hepaticum*.

JACOB KLEIN.

## Microbiology and Parasitology

THE PROGNOSIS IN GONOCOCCAL ENDOCARDITIS. ALBERT B. NEWMAN. *Am. Heart J.* 8:821, 1933.

Gonococcic endocarditis does not necessarily carry with it a fatal outcome. The literature on cases in which recovery was reported to have occurred is reviewed in detail. A case of postabortal gonococcic sepsis with gonococcic endocarditis and peripheral embolic phenomena, focal glomerulonephritis and valvular insufficiency which developed under observation and in which the patient underwent spontaneous recovery is reported.

AUTHOR'S SUMMARY.

GONOCOCCAL ENDOCARDITIS. W. W. BRANDES. *Am. J. Dis. Child.* 46:341, 1933.

A case of gonococcal endocarditis of the tricuspid valve in an infant 10 days old is reported. In all probability, the endocarditis was secondary to gonorrheal conjunctivitis. There was an associated patent ductus arteriosus, which may have been associated with the development and localization of the lesion. The child in this case is the youngest patient having gonococcic endocarditis reported. A report of endocarditis secondary to conjunctivitis could not be found in the literature.

AUTHOR'S SUMMARY.

GONOCOCCIC MENINGITIS IN A NEW-BORN INFANT. W. L. BRADFORD and H. W. KELLEY. *Am. J. Dis. Child.* 46:543, 1933.

In a new-born infant normally delivered from a mother with gonococcic cervicitis ophthalmia neonatorum developed on the second day. On the sixth day, generalized illness occurred, including fretfulness, fever, refusal of food and swelling and redness of the joints. On the eighteenth day, a diagnosis of purulent meningitis was established. From the spinal fluid an organism was isolated in pure culture which on further study gave the morphologic, cultural, fermentative and agglutinating characteristics of the gonococcus. The infant died on the forty-first day, and autopsy revealed purulent meningitis. The same organism was isolated from the meningeal exudate. In view of the clinical and bacteriologic evidence, a diagnosis of gonococcic meningitis seems justified.

AUTHORS' SUMMARY.

CULTURE OF THE TUBERCLE BACILLUS BY THE LÖWENSTEIN METHOD. C. W. LAYMON. *Arch. Dermat. & Syph.* 28:35, 1933.

Laymon cultured tubercle bacilli on Löwenstein's, Korper's and Dorset's mediums and concluded that Löwenstein's medium was superior to the other two. One hundred and forty specimens of blood from thirty-five patients with tuberculosis, tuberculids, lupus erythematosus, erythema induratum, erythema nodosum and erythema multiforme were cultured according to Löwenstein's method and on his medium. All the cultures were negative. Laymon emphasizes the difficulties of the procedure, the expense involved and the time required. He is unable to explain the entirely negative results.

S. W. BECKER.

SPOROTRICHOTIC CHANCRE. H. S. CAMPBELL, K. FROST and O. A. PLUNKETT. *Arch. Dermat. & Syph.* 28:61, 1933.

A small lesion over the dorsum of the distal interphalangeal joint following a scratch healed slowly and then broke down and remained for five months. Cultures of the seropus on Sabouraud's medium gave no growth. Some of the tissue placed on Sabouraud's medium gave a growth of *Sporotrichum*. The organism was inoculated intraperitoneally and subcutaneously in several rats and guinea-pigs, and in only one rat could it be recovered from a subcutaneous lesion. The authors emphasize the difficulty of recovering *Sporotrichum* from some lesions.

S. W. BECKER.



FOCAL CELL REACTIONS IN TUBERCULOSIS AND ALLIED DISEASES. THEOBALD SMITH, Bull. Johns Hopkins Hosp. **53**:197, 1933.

"The idea presented in the foregoing pages is the stimulating activities of the tubercle bacillus. This may manifest itself in many grades of intensity, leading to the development of epithelioid cells, giant cells, the stimulation of fibroblasts, and possibly the direct production of necrosis in high concentration. The epithelioid cell is not related to the macrophage either as to origin or function. It is formed locally through contact of its progenitors with parasites or their products. It differs in these respects from polymorphs which are frankly phagocytic. Their activity amounts to a protection of the host generally, whereas the progenitors of the epithelioid and foreign body giant cells tend to perform this function locally. This idea is in contrast with the concept of phagocytosis which postulates prompt specific aggressiveness on the part of tissue cells. The idea implies, moreover, that immunity tends towards lesser and lesser susceptibility on the part of the cells instead of greater activity, and ending finally in the feeblest reaction of the foreign body giant cell. The change following primary infection as manifested in secondary foci is assumed to be associated with antigenic and antibody products discharged from the primary foci which act upon the bacilli in transit and prepare the reticulo-endothelial cells for the secondary encounter. Finally it should be emphasized that differences in the histological structure of tuberculous lesions appearing more or less regularly may imply different races of bacilli as well as genetic or acquired differences in the resistance of individuals of the host species. A study of such variations among tubercle bacilli is still in its beginnings."

LYMPHOGRANULOMA INGUINALE. H. M. COLE, J. A. M. A. **101**:1069, 1933.

Lymphogranuloma inguinale is a distinct granulomatous entity involving the lymph nodes and is generally venereal in origin. After incubation for from one to several weeks, and not necessarily manifested by a primary sore, the disease results in the formation of a chronic bubo which eventually suppurates. In the female and rarely in the male the lymph nodes around the lower portion of the rectum may be involved, the inflammatory reaction often resulting in stricture of the rectum. Occasionally in the female there may be involvement of the lower part of the vaginal wall and of the labia in the form of a chronic ulcerative elephantiasis—esthiomene. The cause of lymphogranuloma inguinale is a filtrable virus which can be transferred to several of the lower animals (monkeys, rabbits, white mice, guinea-pigs). A specific diagnostic cutaneous reaction (Frei reaction) has been evolved, an emulsion of material from unbroken involved nodes being used as the antigen. A series of positive Frei reactions were obtained in patients suspected of having had the disease in the past. Among them were two who had buboes thirty years before, and one who had a bubo between thirty and forty years before. This allergy of the skin apparently persists through life. Thirty-seven persons with lymphogranuloma inguinale and bubo formation, as well as two with esthiomene and thirteen with anorectal symptoms and stricture of the rectal wall, gave positive Frei reactions and many had no history or signs of syphilis or of tuberculosis.

FROM AUTHOR'S SUMMARY.

LYMPHOMATOSIS, MYELOMATOSIS AND ENDOTHELIOMA OF CHICKENS CAUSED BY A FILTERABLE AGENT. J. FURTH, J. Exper. Med. **58**:253, 1933.

A new transmissible strain of leukemia of chickens is described that causes (a) lymphomatosis with or without formation of tumors and with or without leukemia, (b) myelocytomatosis with or without leukemia and (c) endothelioma. All these diseases are transmissible by material free from viable cells, and the available evidence indicates that they are caused by a single filtrable agent.

AUTHOR'S SUMMARY.

CORYZA OF THE DOMESTIC FOWL. J. B. NELSON, J. Exper. Med. **58**:289 and 297, 1933.

By a method combining filtration and cultivation, an unidentified gram-negative bacillus was isolated from the nasal exudate of fowl experimentally infected with an uncomplicated coryza. Isolation was accomplished by cultivation of the nasal exudate on sealed blood agar plates after unsuccessful attempts to produce colonies on open plates. Injection of the organism into the palatine cleft of a normal bird was regularly followed by an inflammation of the nasal mucosa and a discharge from the nares. A para-influenza bacillus which was also recovered from the nasal tracts of affected fowl was innocuous. Certain cultural characters of the bacillus, bearing on its classification, are discussed.

Three types of an uncomplicated fowl coryza, differing in onset and duration of symptoms, developed after nasal exudate from fowl naturally infected was injected intranasally into normal birds. With two of the types protection tests were carried out in an attempt to explain why the "bacilliary" disease regularly ran a shorter course than the "exudate" disease. Reciprocal protection was demonstrated in one case, but in the other the birds which had recovered from the "bacilliary" disease were susceptible to reinfection from the exudate. There was no indication, however, that a second infectious agent was present in the exudate, and the failure to cross-immunize was ascribed, rather, to a reduction in the immunizing properties of the specific bacillus induced by artificial cultivation. It was also noted that the coryzas produced by injection of exudate and bacilli, respectively, could be transmitted from infected birds to normal ones by direct contact. In both cases one bird out of five failed to contract coryza on exposure. When the respective agents to which these two birds had been exposed were later injected into them, the birds were found to be resistant.

AUTHOR'S SUMMARIES.

THE EFFECT OF A PRIMARY INFECTION ON CONTACT TUBERCULOSIS IN RABBITS. M. B. LURIE, J. Exper. Med. **58**:305, 1933.

Seventy-three per cent of normal rabbits exposed for about one year to cage mates infected with tubercle bacilli of bovine type acquired respiratory or alimentary tuberculosis, which was fatal in 50 per cent of the cases; 63.6 per cent contracted tuberculosis during the first six months. Of rabbits vaccinated with tubercle bacilli of human type and exposed in the same cages at the same time, only 36.8 per cent acquired tuberculosis during the first six months. Later this resistance waned, and by the end of the year altogether 60 per cent had contracted tuberculosis, of which 38 per cent succumbed. The disease in the vaccinated rabbits was shown to be of exogenous origin by the isolation in pure culture from the same rabbit of the human type of bacillus from the primary infection, and of the bovine type of bacillus from the naturally acquired lesion. The vaccination reduced the incidence, extent and mortality of the disease, affected the route of infection, changed its pathologic character, and retarded its progress. The disease acquired by vaccinated rabbits shared many characteristics with the type of tuberculosis found in adult human beings. It is suggested that this method may be used with relative ease in studying many phases of naturally acquired tuberculosis in small laboratory animals.

AUTHOR'S SUMMARY.

THE PARTICULATE NATURE OF THE BACTERIOPHAGE. R. F. FEEMSTER and W. F. WELLS, J. Exper. Med. **58**:385, 1933.

Experimental evidence is brought forward to show that the bacteriophage obeys very closely the laws of chance distribution of particles in suspension.

AUTHORS' SUMMARY.

PSEUDORABIES (INFECTIOUS, BULBAR PARALYSIS, MAD ITCH). E. W. HURST, J. Exper. Med. 58:415, 1933.

The histologic aspects of pseudorabies differ materially in various animal species. In the rabbit, subcutaneous, intradermal or intramuscular inoculation leads to local inflammation and necrosis. The infection ascends the peripheral nerves (possibly both interstitially and by the axis-cylinders) to the corresponding spinal ganglions and segments of the spinal cord, where primary degeneration of nerve and glial cells takes place. The nerve cell changes are probably responsible for the cardinal symptom of the disease, itching. Death ensues soon after the virus reaches the medulla, before visible changes have been produced there. Intracerebral inoculation is followed by characteristic lesions in the meninges, in subpial glial cells and in superficially placed nerve cells. Morbid changes in the lungs are not necessarily related to the presence of the virus, but specific lesions may be present. Intracellular inclusions bearing some resemblance to those seen in herpetic encephalitis, yellow fever, etc., occur in cells derived from all embryonic layers. The disease in the guinea-pig resembles closely that in the rabbit and is modified only by the slightly greater resistance of the guinea-pig. In the monkey, after intracerebral inoculation, widespread degeneration and necrosis of cortical nerve cells occur and are accompanied by specific nuclear alterations in nerve and glial cells but not in cells of mesodermal origin. No lesions are found in other viscera. In the spontaneous disease in the cow the lesions approximate those in the monkey more closely than those in the rabbit. In the pig, vascular and interstitial lesions predominate, degeneration of nerve cells is relatively slight and typical inclusions are not observed. These differences probably explain the benign course of the malady following subcutaneous inoculation in this animal. The lymphatic system also participates in the reaction to the virus.

AUTHOR'S SUMMARY.

INTRANASAL VIRULENCE OF PNEUMOCOCCI FOR MICE. L. T. WEBSTER and A. D. CLOW, J. Exper. Med. 58:465, 1933.

Smooth colony pneumococci freshly isolated from human beings and instilled in small doses into the nasal passages of special mice raised under standard conditions brought about a characteristic infection, and this spread to healthy contacts, inciting in them either a fatal infection or development of the carrier state. Differences in the response of individual hosts to the same dose of a given culture ranged from a completely refractory or nasopharyngeal carrier state, or a local cervical lymphadenitis, to fatal lobular or lobar pneumonia with or without pleurisy, empyema and pericarditis, and acute fatal septicemia. The pneumococci exhibited consistent individual strain differences with respect to ability to infect when instilled intranasally into mice, and also differences in spread to contacts. Degree of intranasal virulence paralleled demonstrable ability to spread to contacts. Degree of intranasal virulence of strains did not parallel degree of intraperitoneal virulence in 50 per cent of the strains—high intranasal virulence was accompanied by either high or moderate intraperitoneal virulence, and low intranasal virulence by high, moderate, or low intraperitoneal virulence. Type III strains were of relatively high intranasal and intraperitoneal virulence; type II strains were mostly of low intranasal but of high or moderate intraperitoneal virulence. Most strains of other types were of low intranasal as well as low intraperitoneal virulence. The intranasal virulence of the pneumococci was not enhanced by passage through animals. Nasal passage reduced the intranasal virulence to zero but did not affect the intraperitoneal virulence, form of colony and agglutinative specificity. Intraperitoneal passage maintained the characteristic level of the intranasal virulence for a period, increased the intraperitoneal virulence in some instances, but did not affect the form of colony or the agglutinative properties.

AUTHORS' SUMMARY.

THE FIBRINOLYTIC ACTIVITY OF HEMOLYTIC STREPTOCOCCI. W. S. TILLET and R. L. GARNER, *J. Exper. Med.* **58**:485, 1933.

Broth cultures of hemolytic streptococci derived from patients are capable of rapidly liquefying normal human fibrin clot. The active fibrinolytic principle is also contained in sterile cell-free filtrates of broth cultures. The degree of activity of the filtrates parallels the activity of the whole broth cultures sufficiently closely to indicate that large amounts of the fibrinolytic substance are freely excreted into the surrounding medium and pass readily through Berkefeld V, Seitz and Chamberland filters. The occurrence of fibrinolysis is most strikingly observed when plasma or fibrinogen is mixed with active cultural material before the formation of the clot is effected. Under the standard experimental conditions described, complete dissolution of human plasma clot (whole oxalated plasma plus calcium chloride) occurs in about ten minutes; complete dissolution of human fibrinogen clot (chemically isolated fibrinogen minus the thrombin) takes place in about two minutes. Titrations of the activity of the filtrates are recorded in table 4 in the article. Twenty-eight strains of *Streptococcus haemolyticus* isolated from patients suffering from various manifestations of streptococcal infection have been tested for capacity to liquefy fibrin clot. Broth cultures of all the strains induced fibrinolysis. Of eighteen strains of *Str. haemolyticus* of animal origin, only three were capable of causing dissolution of fibrin clot. Completely negative results were obtained with thirty-eight strains of other bacterial species. The plasma of many patients who had recovered from acute infections with hemolytic streptococci, when clotted in the presence of active cultures, was highly resistant to fibrinolysis. Furthermore, serum derived from patients whose plasma clot was resistant often conferred on normal plasma clot an antifibrinolytic property. One example of the resistance possessed by the blood of convalescent patients is presented in this report. A second article, now in preparation, will give in detail a large number of observations on the relation of infection to the development of resistance to the fibrinolytic activity of hemolytic streptococci. In contrast to the susceptibility of normal human fibrin clot, rabbit fibrin clot is totally resistant to dissolution when tested under comparable conditions. The insusceptibility of rabbit fibrin clot is manifest provided the coagulum is composed of rabbit constituents. When human thrombin is used to clot rabbit plasma or fibrinogen in the presence of active cultures, fibrinolysis is not prohibited. The rôle of thrombin in determining the resistance or susceptibility of rabbit fibrin to dissolution offers a suggestive approach to problems relating to the underlying mechanism.

AUTHORS' SUMMARY.

THE RELATION OF VITAMIN C DEFICIENCY TO INTESTINAL TUBERCULOSIS IN THE GUINEA-PIG. M. MCCONKEY and D. T. SMITH, *J. Exper. Med.* **58**:503, 1933.

Seventy-two adult guinea-pigs were fed tuberculous sputum daily for periods ranging from six weeks to four months. Thirty-seven of these were maintained on a diet partially deficient in vitamin C; twenty-six acquired ulcerative intestinal tuberculosis. Of the remaining thirty-five animals, the diet of which was supplemented by an adequate amount of vitamin C, only two acquired tuberculous ulcers in the intestines. From these studies we conclude that the ingestion of tubercle bacilli is not the sole factor in the production of intestinal tuberculosis in the guinea-pig. In our opinion, an adequate supply of vitamin C usually protects the guinea-pig against ulcerative intestinal tuberculosis.

AUTHORS' SUMMARY.

THE TITRATION OF POLIOMYELITIC VIRUS CONTAINING TISSUE. M. BRODIE, *J. Immunol.* **25**:87, 1933.

Rhesus monkeys appear to have a uniform susceptibility to the virus of poliomyelitis and so it is possible to determine the minimal completely paralyzing dose (M. C. P. dose) for cord specimens. In passage of the virus through monkeys the infectivity of cord tissue varied from animal to animal; so, if experimental

results are to be compared, they must be expressed in terms of the M. C. P. dose of virus. Intracerebral inoculation of from 1 to 80 M. C. P. doses did not appear to influence the period of incubation or the course of the disease, but borderline infective doses prolonged both, while overwhelming amounts of virus shortened them. Subinfective intracerebral doses of the virus of poliomyelitis failed either to immunize animals or to render them more susceptible to the virus.

#### AUTHOR'S CONCLUSIONS.

FILTRABLE VIRUS CARRIERS. C. S. GIBBS, *J. Infect. Dis.* **53**:169, 1933.

Filtrable virus carriers are reported in hog cholera, rinderpest and infectious laryngotracheitis of poultry. This is the first instance in which filtrable virus carriers have been identified a priori by laboratory tests and not merely referred to as possibilities through reasoning a posteriori. Studies of this nature are important, for an exact knowledge of carriers is indispensable to any program for the control of disease.

#### AUTHOR'S SUMMARY.

TUBERCLE BACILLUS DISSOCIANTS AND VARIANTS. F. B. SEIBERT, E. R. LONG and N. MORLEY, *J. Infect. Dis.* **53**:175, 1933.

A typical avian tubercle bacillus (Van Es, 1921) grew in rough, dry colonies on Long's synthetic medium and in smooth, greasy colonies on Petroff's egg medium. The S bacilli were longer, more slender, more beaded and less acid-fast than the R bacilli, and tended to stratify, whereas the R bacilli piled up in irregular loose clumps. In the few experiments carried out, the S bacilli proved more virulent for hens than the R bacilli. Differences were conspicuous in the spleen. Soon after inoculation of the hens with S bacilli numerous minute tubercles formed, which were noncaseous and loaded with bacilli. In hens infected with R bacilli at the same time, tubercles were fewer, larger and caseous, with few visible bacilli. At a later period the lesions were more nearly alike, but more numerous in the hens infected with S bacilli. Chemically the S form of the avian bacillus differed from the R form in several respects. The S bacilli contained less water and more fatty material than the R bacilli, and the fatty extracts of the S bacilli possessed higher iodine numbers. Comparable chemical fractionations were made of two strains of tubercle bacilli of human type and of different virulence, H 37 and R 1, grown on both Long's synthetic medium and Petroff's egg medium. The appearance of the colonies did not differ on the two mediums, but characteristic chemical differences existed as in the case of the two visibly different forms of avian bacilli.

#### AUTHORS' SUMMARY.

MONILIASIS WITH MENINGEAL INVOLVEMENT. L. W. SMITH and M. E. SANO, *J. Infect. Dis.* **53**:187, 1933.

A case of moniliasis involving the meninges in an infant of 22 months is presented. Bacteriologic studies suggested that the organism is of the type of *Monilia albicans* although it presents certain minor cultural, fermentative and serologic variations. Experimental data show that the organism is highly pathogenic for rabbits and in these animals tends to have a curious and unusual neuropathic affinity, producing invariably, on intravenous inoculation, definite meningitis as well as focal lesions in the brain. In this respect, the organism is much more virulent than the control strains, which less regularly produce involvement of the central nervous system.

#### AUTHORS' SUMMARY.

BACTERIOPHAGE OF *BACILLUS PERTUSSIS*. L. W. SAUER and L. HAMBRECHT, *J. Infect. Dis.* **53**:197, 1933.

The bacteriophage of *Bacillus pertussis* was produced by growing young cultures of old strains of *B. pertussis* in a concentrated fresh sewage bouillon which contained a small amount of defibrinated blood. Lysis was transmitted in series, and platings made from such lysed tubes gave evidence of plaques.

#### AUTHORS' SUMMARY.

MICROMOTION PICTURES OF THE GROWTH OF MYCOBACTERIUM PHLEI. R. W. G. WYCOFF and K. C. SMITHBURN, J. Infect. Dis. **53**:201, 1933.

Micromotion pictures have been made of the development of the timothy grass bacillus, *Mycobacterium phlei*, on solid mediums. In the phase of active growth the bacteria are rods which multiply only by transverse fission. As they age, the rods fractionate into coccobacilli, many of which eventually round up into deeply staining acid-fast cocci. These cocci apparently represent a resting stage of the organism. On a fresh medium they (1) elongate into bacilli, (2) sprout into rods smaller in diameter than the spheres from which they spring or (3) through coccoid division produce smaller cocci that eventually become rods. There is no evidence that in *M. phlei* these growth changes are repetitively cyclic.

AUTHORS' SUMMARY.

GONOCOCCIC MENINGITIS. M. M. STRUMIA and J. J. KOHLHAS, J. Infect. Dis. **53**:212, 1933.

Involvement of the nervous system by gonococcic infection appears to be rare. There are in the literature only seven approved cases of gonococcic meningitis, including our own. In addition to these, ten other cases have been reported as cases of gonococcic meningitis in which that diagnosis is probably correct. Even if the seventeen cases are all accepted as authentic, they are too few to be treated statistically. All proved cases of gonococcic meningitis occurred in the course of acute gonorrheal infection or during acute exacerbation of chronic gonorrheal infection. Only two of the seventeen cases occurred in women. The ages of the patients were from 9 to 30 years, the majority of the cases occurring in patients between 18 and 23 years of age. In most of the instances, other and more common complications of gonorrheal urethritis occurred before, during or after the meningeal symptoms, such as prostatitis, cystitis, arthritis, endocarditis and cutaneous exanthem (purpura). In a few instances, however, the meningitic signs were the first to appear in addition to the urethritis. Such was the case in our patient. In all likelihood, the meningitis follows gonococcemia and is only an uncommon localization of a common generalized condition. In two of the seven proved cases the patients died, and in only one was an autopsy made. The post-mortem observations were similar to those of meningococcic meningitis. If we include the probable but not proved cases, six of a total of seventeen terminated in death. The therapeutic measures employed appear to be of so varied a nature as to make their value doubtful. It can be stated, however, that repeated spinal punctures and drainage offer considerable relief from pain and discomfort. It appears to be worth while to make a careful bacteriologic study in cases of meningitis complicating acute gonorrheal urethritis.

AUTHORS' SUMMARY.

EFFECT OF FEEDING SALMONELLA ORGANISMS TO RATS. E. VERDER, E. DOWNING and H. W. WILCOX, J. Infect. Dis. **53**:245, 1933.

Living *Salmonella aertrycke* and *Salmonella enteritidis*, when eaten, caused diarrhea in varying percentages of white rats, both those on balanced diets and those on diets containing more than a normal amount of starch, sugar or protein. The symptoms rarely appeared in less than twenty-four hours after the feeding. They would seem, therefore, to result from the multiplication and probable rapid disintegration of the swallowed organisms.

AUTHORS' SUMMARY.

SALMONELLA FOOD POISONING. E. VERDER and C. SUTTON, J. Infect. Dis. **53**:262, 1933.

Two persons who had eaten heated custard cultures of a recently isolated strain of *Salmonella enteritidis* and five persons who had drunk heated or filtered washings from similar cultures did not become ill, though in one case the heated washings contained a few living organisms. Another person who ate a custard

culture of another enteritidis strain which had been insufficiently heated to kill many of the contained organisms became definitely ill, with diarrhea developing in twenty hours and marked prostration in thirty-six hours. Monkeys likewise became ill only when living organisms (*S. enteritidis*) were taken. Their symptoms were strikingly similar to those of human beings with food poisoning despite variations in the severity of the reactions and lack of uniformity in the positive results.

AUTHORS' SUMMARY.

RELATION OF SPIRAL ORGANISMS TO THE ROUGH COLONY OF BACTERIUM FUSIFORMIS. R. TUNNICLIFF, *J. Infect. Dis.* **53**:280, 1933.

This study of the colonies of *Bacterium fusiforme* shows that colonies containing the bacillary forms are smooth, and that the spiral organisms are dissociants of the fusiform bacillus, associated with its rough colony.

AUTHOR'S SUMMARY.

ROCKY MOUNTAIN SPOTTED FEVER AND BOUTONNEUSE FEVER. L. F. BADGER, *Pub. Health Rep.* **48**:507, 1933.

"Boutonneuse fever of the Mediterranean littoral, first described by Conor and Bruch, is an acute, noncontagious, febrile disease transmitted by the tick *Rhipicephalus sanguineus* and characterized clinically by fever, headaches, muscular and joint pains, and an exanthem." Brumpt concluded on immunologic grounds that boutonneuse fever and Rocky Mountain spotted fever are distinct entities. Badger's tests indicate that they are immunologically identical.

THE RELATIONSHIP BETWEEN ZOSTER AND VARICELLA AS SHOWN BY COMPLEMENT FIXATION. R. T. BRAIN, *Brit. J. Exper. Path.* **14**:67, 1933.

The serums of persons convalescent from herpes zoster and varicella contain specific antibodies, which can be demonstrated by complement fixation when the vesicle fluids are used as antigens. The vesicle fluid of herpes zoster gives equally good fixation in the presence of both herpes zoster and varicella antibodies, and the same is true of the vesicle fluid of varicella. These findings are in conformity with those of Netter and his colleagues. They indicate the close relationship and possible identity of the viruses of herpes zoster and varicella.

AUTHOR'S SUMMARY.

A VITAMIN NECESSARY FOR THE GROWTH OF *B. SPOROGENES*. C. J. G. KNIGHT and P. FILDES, *Brit. J. Exper. Path.* **14**:112, 1933.

A factor, comparable to a vitamin, necessary for the growth of *Clostridium sporogenes* is described. Its chemical nature is indicated. Evidence is brought forward to suggest that this vitamin may be closely allied to other factors which have been described as affecting the growth of plants and other forms of life.

AUTHORS' SUMMARY.

NUCLEAR INCLUSIONS IN THE LIVER IN RIFT VALLEY FEVER. G. M. FINDLAY, *Brit. J. Exper. Path.* **14**:207, 1933.

Intranuclear acidophilic inclusions have been found in the hepatic cells of the sheep, goat, rhesus monkey, marmoset, rat, mouse, field vole, wood vole, golden hamster and gray squirrel infected with Rift Valley fever. The inclusions develop in the nucleoplasm of the hepatic cells and are visible in fresh preparations. The inclusions do not give a reaction for fats, thymonucleic acid or masked iron. Their development coincides with a margination of the nuclear chromatin on the nuclear membrane, which eventually ruptures. The morphologic and tinctorial properties of the inclusions are described and compared with those associated with other viruses that give rise to inclusions in the nuclei of the cells of the liver. The lysis of mitochondria and the disruption of the Golgi apparatus in the cells of the liver in Rift Valley fever are also described.

AUTHOR'S SUMMARY.

VARIATION IN STOCK CULTURES OF MENINGOCOCCI. B. G. MAEGRAITH, Brit. J. Exper. Path. **14**:219 and 227, 1933.

Great variation in agglutination by "type" serums occurs among single colonies of stock meningococci. Littledale (type I) strains show a tendency to overlap into type III to such an extent that these types appear to be approaching interchangeability. There is little evidence of a division of group and specific antigens among single colonies derived from stock type cultures. The so-called types I and III of the meningococcus are in all probability not fixed types.

Certain variants of stock strains of meningococci can be detected by the characteristics of their growth on homologous immune serum agar. One variant develops haloes in this medium and the other does not. Investigation of the properties of these variants has indicated that they are of "smooth" and "rough" nature, respectively. This is supported by their differences in growth in various mediums, in their fermentation and precipitin reactions and in their effects on guinea-pigs. Agglutination and absorption reactions with serums prepared from the variants do not agree with the results obtained by Enders with his "S" and "R" forms. A tentative explanation of this discrepancy is offered. These variants are being further investigated.

AUTHOR'S SUMMARIES.

THE SIZE OF THE VIRUS OF FOWL PLAGUE ESTIMATED BY ULTRA-FILTRATION. W. J. ELFORD and C. TODD, Brit. J. Exper. Path. **14**:240, 1933.

The diameter of the elementary particles of the virus of fowl plague as they exist in the body fluids of the fowl has been estimated by analytic ultrafiltration through graded collodion membranes to be from 60 to 90 microns. Control experiments in which Staphylococcus K bacteriophage (size, from 50 to 75 microns) was admixed with a suspension of the virus confirmed the close relationship in size between the particles of this phage and those of the virus. Differentiation of their filtrabilities was only just possible.

AUTHORS' SUMMARY.

DEVELOPMENTAL FORMS OF THE VIRUS OF PSITTACOSIS. S. P. BEDSON, Brit. J. Exper. Path. **14**:267, 1933.

Evidence has been produced to show that the forms described in the early stages of infection of the mouse with psittacosis are developmental stages of the virus. The virulence of the virus in the form of plaques or primary morulae is considerably less than that of the elementary bodies. The large or intermediate forms filter less readily than the elementary bodies, but the latter are thrown down more readily by centrifugation. It would appear that the virus of psittacosis produces inclusions, though not the fully developed acidophil inclusions characteristic of many viruses. It has been found impossible to say whether the plaque is homogeneous—a plasmodium—or an aggregate of virus particles the detail of which is obscured by inclusion material.

AUTHOR'S SUMMARY.

THE FILTRATION OF SPIROCHAETES THROUGH GRADED COLLODION MEMBRANES. E. HINDLE and W. J. ELFORD, J. Path. & Bact. **37**:9, 1933.

Filtration experiments in which graded collodion membranes were used showed that the limiting porosity for *Spirochaeta biflexa* is one in which the pores measure 0.25 micron and that the porosity for *Spirochaeta pallida* is one in which the pores measure approximately 0.4 micron. This is interpreted as indicating a diameter of 0.1 micron for the former and a diameter of 0.2 micron for the latter. The corresponding figures obtained from photographs made by the method in which an ultraviolet ray background is used were approximately 0.11 and 0.33 micron. The spirochetes passed readily through the membranes. This method furnishes a simple and effective means of separating spirochetes, including *S. pallida*, from other organisms. No evidence was obtained of the existence of a special filtrable phase with a diameter significantly smaller than that of the spirochete.

AUTHORS' SUMMARY.



## Immunology

A DIAGNOSTIC TEST FOR INFECTIOUS MONONUCLEOSIS. W. W. BUNNELL, Am. J. M. Sc. **186**:346, 1933.

We have employed the sheep cell agglutinin test in over two thousand cases representing seventy-six clinical conditions. With the exception previously noted, namely, serum disease, we have been unable to demonstrate an appreciable increase of heterophile agglutinins for sheep cells in the serums above the normal dilution of 1:8. In fifteen cases of infectious mononucleosis we have found a consistent increase in all the cases. The titers, ranging from 1:64 to 1:4,096, apparently depended to a considerable extent on the stage of the disease at which the serum was obtained and on the severity of the illness. None of the common conditions manifesting a similar clinical picture, such as acute adenitis, tuberculous or syphilitic adenitis, Hodgkin's disease, acute or chronic lymphatic or myelogenous leukemia, aplastic anemia, purpura haemorrhagica, agranulocytic angina or Vincent's angina, has shown an increase in heterophile agglutinin titer. In view of these observations it seems justifiable to accept the test for heterophile agglutinins for sheep cells as a valuable diagnostic procedure in differentiating infectious mononucleosis from a number of clinical conditions of a far more serious nature. Cases presenting a suggestive clinical and serologic picture and blood serum with an agglutination for sheep cells in a dilution of at least 1:64 can apparently safely be diagnosed as infectious mononucleosis and a favorable prognosis given.

AUTHOR'S SUMMARY.

THE COMPARATIVE SENSITIVENESS OF THE PIRQUET AND THE INTRACUTANEOUS TUBERCULIN TESTS. J. D. ARONSON, D. ZACKS and J. J. POUTAS, Am. Rev. Tuberc. **27**:465, 1933.

Two large groups of white persons near Boston were tested simultaneously by both methods. An average of 2.9 per cent of a group of children between the ages of 5 and 19 years who failed to react to the Pirquet test reacted to 0.01 mg. of tuberculin. When 1 mg. was employed, the percentage of those who failed to react to the Pirquet test but reacted to the intracutaneous test averaged 19.1. The total number of those who reacted to the intracutaneous test and not to the Pirquet test revealed a negligible increase in the amount of infection detected by this means. Repetition of the Pirquet test yielded a negligible increase. A mathematical factor, a "correction figure," could not be devised to convert results by the one method into those obtained by the other. The intracutaneous test can be carried out as expeditiously as the Pirquet test. The intracutaneous tuberculin test is more sensitive than the Pirquet test in determining the incidence of tuberculous infection. Results obtained by the two methods are not directly comparable.

H. J. CORPER.

THE PERSISTENCE OF IMMUNITY AFTER THE ABOLITION OF ALLERGY BY DESENSITIZATION. A. R. RICH, F. B. JENNINGS, JR., and L. M. DOWNING, Bull. Johns Hopkins Hosp. **53**:172, 1933.

Rich and his associates carried out experiments on *Pasteurella aviseptica* and pneumococcic infection in order to determine whether immunity would be less efficient after the abolition of allergy by desensitization. When the capacity for reacting with allergic inflammation is completely destroyed by the injection of large doses of killed bacteria into the veins of hypersensitive, immune animals, immunity to intracutaneous infection with millions of lethal doses of living virulent bacteria remains intact. Inflammation at the site of infection in such animals is far less than in untreated allergic, immune animals—far less, indeed, than in normal controls; but the bacteria are prevented from spreading from the site and are as effectively destroyed as in the allergic, immune body. The immune state so greatly enhances the protective power of the inflammatory mechanism that much

less, rather than much more, inflammation is required to protect the body from infection. Damage to the tissue at the site of infection is also far less in desensitized animals than in allergic ones. Even in the highly allergic, immune body, therefore, allergic inflammation is not necessary for protection against infection, and in its absence the body is protected with much less damage to the tissue than that which occurs in the allergic body.

AUTHORS' SUMMARY.

PNEUMOCOCCUS AUTOLYSATES IN DERMAL INFECTION IN RABBIT. K. GOODNER, J. Exper. Med. 58:153, 1933.

In dermal infections caused by the pneumococcus in rabbits, the addition of pneumococcus autolysate to an infective inoculum favors the invasiveness of the particular strain employed but does not alter the kind of virulence possessed by that strain. Autolysates exhibiting this enhancing property also induce purpura in mice and inhibit the coagulation of blood in rabbits. The relation of these properties to the infectivity of the pneumococcus and the possible rôle of bacterial autolysis in natural infection are discussed.

AUTHOR'S SUMMARY.

SPREADING BACTERIAL FACTOR. F. DURAN-REYNALS, J. Exper. Med. 58:161, 1933.

Invasive strains of staphylococci and streptococci contain a soluble factor which markedly increases the permeability of tissue and enhances the infections produced by these organisms, by other bacteria and by vaccine virus as well. The non-evasive strains of the same species of staphylococci and streptococci do not contain this factor. The enhancing substance elaborated locally by organisms passes into the circulating blood and may enhance local infections elsewhere by bringing about a general increase of the permeability of tissues. In the phenomena it elicits, the factor is similar to the spreading factor extracted from many animal tissues, especially from the testicle.

AUTHOR'S SUMMARY.

RESISTANCE TO PNEUMOCOCCUS INFECTION IN RABBITS. K. GOODNER and E. G. STILLMAN, J. Exper. Med. 58:183 and 195, 1933.

The dermal pneumococcic infection was employed to determine the active resistance of rabbits against infection, and an evaluating scale of the gradient of resistance was established. The significance of the various degrees of resistance is discussed, and the possible general inferences of the observations are indicated.

The degree and duration of the active resistance against pneumococcic infection were determined in rabbits which received intravenous injections of a heat-killed suspension of pneumococci of the three principal types. The resistance induced by the immunization procedure varied in degree and in duration with each type of pneumococcus.

AUTHORS' SUMMARY.

VACCINATION WITH HEAT-KILLED AND FORMALDEHYDIZED TUBERCLE BACILLI. R. M. THOMAS, J. Exper. Med. 58:227, 1933.

Rabbits vaccinated with tubercle bacilli killed by exposure to a solution of formaldehyde (0.4 per cent) did not show any acquired resistance to subsequent infection with bovine tubercle bacilli, while rabbits vaccinated with tubercle bacilli which had been killed by heating to 70 C. for one hour survived more than half as long again as their controls. Intraperitoneal injection of either the formaldehyde-killed or the heat-killed vaccine into guinea-pigs made them skin-sensitive to human tubercle bacillus protein. The rate of absorption of the formaldehyde-killed vaccine when introduced beneath the skin was similar to that of the heat-killed vaccine. Following the intravenous injection of heat-killed tubercle bacilli, it was found that a massive tuberculous pneumonia developed in rabbits. A study of the production and ultimate absorption of the cellular exudate showed that these processes were

similar to those found after the injection of living bacilli. The lesions which followed the injection of heat-killed bacilli differed from the lesions found in active tuberculosis in that in any one animal they showed a striking uniformity of appearance, while in the active disease the lungs showed a great diversity in type of lesion. Studies of the blood cells during the period of injection of dead organisms showed that the changes which are characteristic of the period during which a tuberculous pneumonia develops in rabbits (from thirty to forty days after inoculation) were faithfully reproduced. It is suggested that the process of regression described may be similar to that which occurs in tuberculosis of childhood, in which rather extensive pulmonary lesions resolve without leaving evidence of damage to the parenchyma of the lung.

AUTHOR'S SUMMARY.

#### EFFECT OF NONSPECIFIC AGENTS ON ACTION OF ANTIPNEUMOCOCCUS SERUM.

A. G. GELARIE and A. B. SABIN, *J. Exper. Med.* **58**:237, 1933.

Gelarie and Sabin attempted to determine whether or not nonspecific agents are capable of exerting any influence on the response of animals infected with the pneumococcus to specific serum therapy. They demonstrated that whereas gold (empirically chosen) by itself had very little effect on either the course or the outcome of the experimental pneumococcal infection, it is nevertheless capable of exerting a definite and marked beneficial effect in rabbits treated with a sub-effective dose of the specific antiserum. Of the rabbits treated with the subeffective dose of serum alone 71 per cent died and only 29 per cent survived. The additional administration of gold reversed this ratio with the result that in a large group of rabbits which received the combined therapy, 77 per cent survived and only 23 per cent died.

AUTHORS' SUMMARY.

#### CHANGES IN THE TITER OF ANTI-PNEUMOCOCCAL HUMORAL IMMUNITY IN

ADULT HUMAN BEINGS. J. B. GRAESER and M. C. HARRISON, *J. Exper. Med.* **58**:245, 1933.

Fifty-five persons were tested to determine the pneumococcal promoting activity of their serum against pneumococci of types I and II. By repeated tests an attempt was made to study the constancy of the degree of their immunity over intervals of from two to six months. In this group were included nine persons with common colds and twelve with a severe influenza-like infection. Fifteen of the fifty-five persons tested showed a change in titer of their humoral immunity against either type I or II or both. Three of these showed an increase, and twelve a decrease. In most instances this reaction was a specific one in that the altered reaction toward one type was not associated with a similar change toward the other type of pneumococcus. Colds and influenza-like infections apparently exerted no effect on the titer of humoral immune substances.

AUTHORS' SUMMARY.

#### STREPTOLYSINS. B. E. HODGE and H. F. SWIFT, *J. Exper. Med.* **58**:277, 1933.

Certain properties of streptolysin in respect of hemolytic power and capacity for combining with antistreptolysins have been determined. The hemolytic strength may vary markedly, while under suitable conditions the combining power is constant. This stability of combining makes it possible to prepare a large amount of streptolysin, standardize it against serums of known antistreptolysin strength, and use it as a reagent over a considerable period for testing the antistreptolysin content of unknown serums. A modified technic for making these tests is described.

AUTHORS' SUMMARY.

#### VACCINATION OF RABBITS WITH PARTIALLY DEFATTED TUBERCLE BACILLI.

K. C. SMITHBURN, *J. Exper. Med.* **58**:329, 1933.

A vaccine prepared from partially defatted bovine tubercle bacilli favorably influenced the survival time after inoculation with living virulent bovine tubercle bacilli. Accompanying this increased resistance was a sustained lymphocytosis in

the vaccinated animals. The vaccine induced a transitory anemia and leukopenia. A similar preparation of partially defatted human tubercle bacilli possessed the power to sensitize guinea-pigs to tuberculo-protein.

AUTHOR'S SUMMARY.

THE ANTIGENIC COMPLEX OF THE MENINGOCOCCUS. G. RAKE and H. W. SCHERP, J. Exper. Med. 58:341 and 361, 1933.

Three fractions have been isolated from autolysates of the meningococcus. Of these, one, the type-specific substance, has been described in detail. The same type-specific substance appears to be present in organisms of types I and III, but a substance differing at least serologically has been obtained from strains of type II.

Two group-specific substances, one a polysaccharide and the other a protein, have been isolated from the meningococcus. Their serologic properties are described.

AUTHORS' SUMMARY.

MENINGOCOCCUS PRECIPITINOGENS IN THE CEREBROSPINAL FLUID. G. RAKE, J. Exper. Med. 58:375, 1933.

Precipitin tests on the cerebrospinal fluid from cases of meningococcal meningitis with monovalent serums demonstrate the presence of type-specific precipitinogens of the meningococcus. Negative results are secured when the spinal fluid is obtained after the commencement of intrathecal serum treatment and also occasionally when the numbers of infecting organisms are very small. The reaction offers an easy and rapid method of ascertaining to which type of meningococcus a particular case of meningitis is due, and facilitates the immediate use of monovalent therapeutic antimeningococcus serum. Typing by means of the precipitin reaction can be confirmed by agglutination of the strain of organism responsible for the infection, if such a strain is isolated. Confirmation by means of agglutination has been possible in all the cases discussed in this report. Spinal fluids from patients with other diseases of the meninges and central nervous system fail to give any precipitin reaction with the monovalent serums.

AUTHOR'S SUMMARY.

THE SPECIFICITY OF ALLERGY TO CERTAIN NEISSERIAE. C. P. MILLER and R. CASTLES, J. Exper. Med. 58:435, 1933.

By means of the reaction to intracutaneous inoculation with bacterial suspensions in amounts of 0.1 cc., bacterial allergy was demonstrated in rabbits into which agar foci containing gonococci, meningococci, *Micrococcus catarrhalis*, or *Bacterium leptosepticum* had been implanted. The criterion of hypersensitiveness is the relative size and intensity of reaction evoked by an identical dose in "agar focus" and control rabbits. Rabbits sensitized to gonococci or meningococci usually reacted indistinguishably to either of these organisms, but were less allergic to *M. catarrhalis*. Similarly, animals sensitized to *M. catarrhalis* gave moderate but not maximal responses to gonococci and meningococci. Cross-reactions did not occur between *Bact. leptosepticum* and any of the three cocci. Animals sensitized to the four organisms mentioned reacted no more intensely than did controls to hemolytic streptococci, staphylococci and rough pneumococci. The hypersensitive state was found to begin early in the second week and to end usually by the fourth week, being at its height in most instances from the tenth to the twelfth day. The number of organisms contained in the agar focus determined the success of the sensitization only to the extent that very small and very large inoculations failed to produce the allergic state. In rabbits immunized by the intravenous injection of living organisms cutaneous reactions indistinguishable from those in controls developed. The "secondary rise" of Andrewes, Derick and Swift was rarely observed.

AUTHORS' SUMMARY.

THE INFLUENCE OF TESTICLE EXTRACT UPON THE EFFECT OF TOXINS, BACTERIA AND VIRUSES, AND ON THE SHWARTZMAN AND ARTHUS PHENOMENA. F. DURAN-REYNALS, J. Exper. Med. **58**:451, 1933.

The lesions produced by the Shwartzman and Arthus phenomena, as well as those produced by bacterial toxin and foreign serums in the normal rabbit, are spread by testicular extract over a larger area than would otherwise be covered. With this spreading of the lesions there is a definite reduction in their intensity. Lesions produced by invasive strains of staphylococci in high dilutions or by non-invasive staphylococci at moderate dilutions are definitely lessened in severity or even suppressed by the spreading action of testicular extract. Lesions due to virus are consistently enhanced by the spreading factor, regardless of the dilution.

AUTHOR'S SUMMARY.

NEUTRALIZING ANTIBODIES IN ABORTIVE POLIOMYELITIS. J. D. TRASK and J. R. PAUL, J. Exper. Med. **58**:531, 1933.

The neutralizing antibody content for poliomyelitis virus has been tested with both a human and a passage strain of the virus in serial samples of serums from five mild cases of abortive poliomyelitis, and an increase in this antibody content has been demonstrated in samples of convalescent serum obtained within four weeks of the acute illness.

AUTHORS' CONCLUSION.

TYPE-SPECIFIC SERUM AGAINST HAEMOPHILUS INFLUENZAE. M. PITTMAN, J. Exper. Med. **58**:683, 1933.

Most of the strains of Haemophilus influenzae that cause meningitis are of the same serologic type (b). A horse immunized with a strain of type b gave a highly type-specific serum with marked anti-infectious action in mice and rabbits. The evidence at hand indicates that this serum may have practical value in influenzal meningitis.

THE SOLUBLE SPECIFIC SUBSTANCE OF PNEUMOCOCCUS TYPE I. O. T. AVERY and W. F. GOEBEL, J. Exper. Med. **58**:731, 1933.

The soluble specific substance of Pneumococcus type I is now regarded, tentatively at least, as an acetyl polysaccharide. In this form it accounts adequately for all the serologic phenomena of type specificity of Pneumococcus type I.

AUTHORS' CONCLUSIONS.

TISSUE CULTURE STUDIES ON THE RELATION OF THE TUBERCULIN REACTION TO ANAPHYLAXIS AND THE ARTHUS PHENOMENON. J. D. ARONSON, J. Immunol. **25**:1, 1933.

The growth and migration of cells in explants from the spleen and bone marrow of tuberculous guinea-pigs are inhibited by the addition of tuberculin; those explanted from nontuberculous guinea-pigs sensitized by a single injection of horse serum are not inhibited by horse serum. Migration and growth in similar explants from tuberculous guinea-pigs sensitized to horse serum are not inhibited by the addition of horse serum; they are, on the other hand, inhibited by tuberculin. When the Arthus phenomenon was elicited by injections of horse serum into normal and tuberculous guinea-pigs the tissue cultures were unaffected by the addition of the serum. These experiments on tissue cultures suggest that the mechanism underlying the tuberculin reaction is different from the mechanisms of anaphylaxis and the Arthus phenomenon.

AUTHOR'S SUMMARY.

THE URTICARIAL SKIN REACTIONS IN GUINEA-PIGS. L. S. PILCHER, JR., J. Immunol. **25**:11, 1933.

Cutaneous reactions typical of urticaria and exactly resembling in character and time sequence those described in man by Lewis and Grant were produced in

guinea-pigs by light skin puncture through a drop of 1:100 solution of histamine. The vascular changes preceding and accompanying the urticarial reaction could be accurately observed by looking through the ear of the guinea-pig while the reaction was in progress. "Immediate" anaphylactic cutaneous reactions to antigenic horse serum were produced in sensitized guinea-pigs by light puncture of the skin through a drop of undiluted horse serum. These reactions were typically urticarial, resembling the wheals produced by histamine but having a more gradual and chronic sequence of development. No general anaphylactic reactions of any kind accompanied any of the cutaneous reactions. The anaphylactic sensitivity of all the animals tested was checked by an intracardiac injection of 2 cc. of normal horse serum at the end of the experiment. There appeared to be a fairly definite relation between the presence of general anaphylactic sensitivity and the production or nonproduction of the typical anaphylactic sensitivity and the degree of production of the cutaneous reaction caused by histamine.

AUTHOR'S SUMMARY.

IMMUNIZATION OF RABBITS WITH HEAT-KILLED TUBERCLE BACILLI. J. HUGHES, *J. Immunol.* **25**:103, 1933.

The serum of rabbits vaccinated by repeated injections of heat-killed tubercle bacilli shows a progressive increase in its ability to promote phagocytosis of living tubercle bacilli by polymorphonuclear and mononuclear cells. The close parallel in the bacteriotropic action of the serum on polymorphonuclear and on mononuclear leukocytes indicates that the same antibody is concerned in the phagocytosis of tubercle bacilli by the two types of cell. The earliest manifestation of bacteriotropic action precedes the onset of sensitization, indicated by the cutaneous reaction to an injection of dead tubercle bacilli. Infection with living tubercle bacilli has caused an earlier appearance of bacteriotropic substance than repeated injections of the dead organism, but the titer attained is no higher.

AUTHOR'S SUMMARY.

LOCAL ORGAN HYPERSENSITIVENESS. B. C. SEEGAL, S. SEEGAL and D. KHORAZO, *J. Immunol.* **25**:207 and 221, 1933.

When 0.15 cc. of undiluted egg white is injected into the anterior chamber of the rabbit's eye, traces may be found for as long as eight days. The egg white can never be demonstrated in the serum or the fluid in the anterior chamber of the other eye. The injection of 0.15 cc. of undiluted egg white into the anterior chamber is followed by the appearance of precipitins against egg white in the serum. The precipitins reach a titer of 1:20,000 or more in about nine days. Precipitins may also be found in the aqueous humor of the eye into which egg white has been injected, though they do not always appear there. In only one animal did they reach a titer as great as that of the serum precipitins. Precipitins never appear in the fluid in the anterior chamber before they appear in the serum. Precipitins are never found in the fluid in the anterior chamber of the other eye. When 0.2 cc. of egg white is injected into rabbits in a single dose subcutaneously, intravenously or intracutaneously no precipitins against this antigen appear in the serum. When the same amount of egg white is diluted and injected in four divided doses subcutaneously or intravenously the serum contains precipitins against egg white to the same titer as that obtained by injecting 0.2 cc. of the antigen into the anterior chamber of the eye. The rabbit's eye was passively sensitized by the injection of rabbit anti-egg-white-serum into the anterior chamber only once in nine attempts. If animals which have been locally sensitized in the eye are allowed to rest until no antigen remains in the fluid in the anterior chamber and are then given an injection of a "shocking" dose of antigen intravenously, the eye becomes inflamed. During the period of inflammation the fluid in the anterior chamber of the sensitive eye may contain traces of the antigen. The appearance of antigen in the fluid in the anterior chamber of a reacting eye may furnish an explanation for the continuation of the hypersensitive stage in this organ, since fresh antigen is available for the stimulation of further sensitivity.

In rabbits whose right eye was inflamed by the introduction of glycerin into the anterior chamber, repeated intravenous injections of egg white resulted in a specific local sensitization of the eye to egg white in twelve of twenty-one animals tested. In control rabbits receiving intravenous injections of egg white alone and in other animals whose right eye was injured with glycerin alone the ocular reaction did not develop when the test dose of egg white was injected intravenously. Repeated injections of egg white intravenously in normal animals may bring about a degree of general reactivity causing later injections of egg white to produce bilateral inflammation of the eyes.

AUTHORS' SUMMARY.

**NONSPECIFIC PRECIPITINS FOR PNEUMOCOCCIC FRACTION C IN ACUTE INFECTIONS.** R. ASH, *J. Infect. Dis.* **53**:89, 1933.

Precipitins against the pneumococcic fraction C described by Tillet and his associates appear early in the course of acute infectious disease and disappear shortly after the cessation of the infection. These precipitins are not restricted to pneumococcic infection but may occur in illnesses due to gram-negative as well as to gram-positive organisms. These precipitins have not been noted in syphilitic infection and appear in only small amounts, if at all, in tuberculous disease. In many instances there is a relationship between fever, an increased rate of sedimentation and this phenomenon of precipitation, but the relationship is not constant. The phenomenon is not one of maturation, related to age or to previous infection, since it may be observed in early infancy.

AUTHOR'S SUMMARY.

**AGGLUTINATION TESTS IN THE DIAGNOSIS OF INFECTIOUS ABORTION IN CATTLE.**

C. R. DONHAM and C. P. FITCH, *J. Infect. Dis.* **53**:98, 1933.

The sensitivity of *Bacterium abortus* agglutination antigen for either the test tube or the rapid test method is increased for most agglutinating serums by the addition of either acacia or gelatin to the antigen. It is not uncommon to encounter agglutinating serums having identical test tube titers that do not respond in the same manner when tested with various kinds of rapid test antigen. The rapid and the test tube method should be standardized each by itself and not one on another. The influence of varying concentrations of electrolytes (as such) in the rapid test method of testing appears to be similar, if not identical, with that in the test tube method. At present, the tests cannot be carried out in the complete absence of electrolytes. Heating rapid test *Bact. abortus* antigen does not exert any significant influence on its sensitivity. The coagulum formed on the surface of a boiled rapid test suspension of antigen has antigenic properties indistinguishable from those of the balance of the boiled antigen. There is no evidence to indicate that the removal of the coagulum increases the sensitivity of the antigen. Titers can be varied by changing the amounts of the antigen. The size of the drops delivered with dropper pipets as they have been supplied with commercial rapid test antigens varies more than the limits within which variation in the amounts of antigen does not yield consistent differences in the titers. A mathematical analysis of the titers obtained in rapid tests of bovine serums by different observers and with different preparations of antigen shows significant variations in the results. Atypical clumping of rapid test antigen may occur. The activity of specific agglutinin for *Bact. abortus* is not appreciably influenced by evaporating the agglutinating serum to dryness.

AUTHORS' SUMMARY.

**PRECIPITATED DIPHTHERIA TOXOID.** L. C. HAVENS and D. M. WELLS, *J. Infect. Dis.* **53**:138, 1933.

The precipitation and purification of diphtheria toxoid by potassium-aluminum sulphate are described. Complete precipitation results from the addition of from 1 to 2.5 per cent of alum. There is little or no loss of specific antigen, while nitrogen determinations show that the protein content is reduced to from 13 to 20 per cent of the original amount. The antigenic activity of the precipitate is superior to that

of ordinary toxoid. Subcutaneous injection of 0.1 cc. (1 unit) into guinea-pigs protects them against a subsequent injection of at least 5 minimum lethal doses of toxin, while 5 units of the precipitate has protected guinea-pigs against 450 minimum lethal doses. A single injection of from 5 to 10 units given to children has resulted in negative reactions to the Schick test in 96 per cent of these children. The superior antigenic character of the precipitated toxoid is probably due to its slow absorption with the resultant prolonged immunizing action.

## AUTHOR'S SUMMARY.

IMMUNIZATION OF SHEEP TO THE VIRUS OF POLIOMYELITIS. B. F. HOWITT.  
J. Infect. Dis. 53:145, 1933.

It has been demonstrated that antiviral substances may be produced by repeated inoculations of poliomyelitic virus into a refractory animal such as the sheep. The immunization was accomplished in two animals within five months, not only by giving centrifugated suspensions of virus, but by inoculating the washed acetone precipitate of such a suspension. Serums of comparable potency were produced by each method. Serums obtained from these animals neutralized the 5 per cent monkey passage strain of virus in final dilutions of 1:200, 1:400 and, in the case of sheep, 404, even 1:800, while that from sheep 117, which was killed after three years of immunization, gave a titer of from 1:100 to 1:200. The latter serum was found to retain its potency for at least thirteen months. It also showed as high a titer as the serum from a patient who had recovered from poliomyelitis, while two other human serums failed to give protection in the same dilutions as the sheep serums. Human serums stored from nine to eleven months in the icebox were found to retain the same neutralizing power for the old virus as that formerly observed. Identical results were also noticed with serums obtained one year after an earlier bleeding. Comparative neutralization tests with both immune sheep serum and human convalescent serum, respectively, against an old and a more recently isolated strain of poliomyelitic virus (New York, 1931) gave evidence of a certain modification of the old virus after repeated passage through monkeys. If the laboratory virus can be definitely altered by prolonged passage through a new host, it is advisable to employ a recently isolated strain both for work on immunization and for testing the antiviral power of immune serums.

## AUTHOR'S SUMMARY.

RELATION OF ALLERGY TO GENERAL RESISTANCE IN STREPTOCOCCIC INFECTION.  
B. J. CLAWSON. J. Infect. Dis. 53:157, 1933.

Resistance to streptococcic infection can be developed artificially without producing a measurable amount of hypersensitiveness (allergy). The state of streptococcic allergy bears no necessary relation to general resistance. Allergy as related to general resistance to streptococci seems to be a useless and at times a harmful concomitant phenomenon.

## AUTHOR'S SUMMARY.

HETEROPHILIC ANTIBODIES IN SERUM DISEASE. I. DAVIDSOHN. J. Infect. Dis. 53:219, 1933.

The difference in the development of the heterophilic antish sheep agglutinin and hemolysin, as contrasted with the nonheterophilic antirabbit antibodies, points to the Forssman antigen in the injected horse serum as the responsible antigenic factor.

## AUTHOR'S SUMMARY.

NEUTRALIZATION OF POLIOMYELITIS VIRUS. N. P. HUDSON and W. LITTERER.  
J. Infect. Dis. 53:304, 1933.

The serums of twenty-five normal adult white residents of Nashville, Tenn., were tested for ability to neutralize the virus of poliomyelitis. Twenty-one specimens were virucidal when examined without dilution, and sixteen of these neutral-



ized the virus when tested again in a further dilution of 1:5. An analysis of the age of the donors in relation to the results showed that those of the upper age group (30 to 44 years) had a higher proportion of neutralizing serums and that more specimens were virucidal in the arbitrarily chosen dilution of 1:5, than those of the lower age group (17 to 29 years). Cases of poliomyelitis have occurred in Nashville and in other parts of Tennessee for many years, without, however, causing the appearance of epidemics. Most of the cases occurred in areas of denser population, but even sparsely settled counties reported cases of the disease more or less frequently. Our experimental results are consistent with the epidemiologic features of the disease in Southern states. Before we conclude, however, whether the neutralizing property demonstrated is a specific response to exposure to the virus of poliomyelitis or whether it is a manifestation of serologic ripening with maturation of the person, further observations and experimentation must be made. The high proportion of persons examined whose serum neutralized virus gives a basis for experimentation in the use of such serum in poliomyelitis.

AUTHORS' SUMMARY.

*PRECIPITIN PRODUCTION IN RABBITS FOLLOWING INTRAMUSCULAR INJECTION OF ANTIGEN ADSORBED BY ALUMINUM HYDROXIDE.* L. HEKTOEN and W. H. WELKER, *J. Infect. Dis.* **53**:309, 1933.

In rabbits a single intramuscular injection of aluminum hydroxide carrying a measured quantity of antigen may induce the continuous formation of precipitin for many months. The aluminum hydroxide appears to be gradually replaced by connective tissue. Antigens adsorbed on aluminum hydroxide do not separate on standing and retain their precipitinogenic properties for at least twelve months and probably much longer. Aluminum hydroxide may carry at least ten distinct, specific antigens at the same time, and the rabbit may respond to a single intramuscular injection with the specific production of precipitin for each of the ten antigens.

AUTHORS' SUMMARY.

*SHWARTZMAN PHENOMENON.* H. M. KLEIN, *J. Infect. Dis.* **53**:312, 1933.

Evidence is presented that in patients recovering from systemic gonococcal infections and from typhoid fever specific antitoxins developed spontaneously, as demonstrated by the Shwartzman phenomenon. It appeared highly suggestive that in these patients recovery was related to the formation of antitoxin. The serum of certain patients with previous meningococcal infections and of certain controls was shown to possess meningococcal antitoxin. This was group specific. The capacity of antigenococcal serum to neutralize meningococcus-reacting factors and of anti-meningococcal serum to neutralize gonococcus-reacting factors is a further evidence of the biologic relationship between the gonococcus and the meningococcus. These investigations are being extended to the other diseases caused by bacteria possessing toxins potent in eliciting the Shwartzman phenomenon. The aim will be to study whether spontaneous formation of antitoxin takes place. If further studies are fruitful, attempts should be made to confer immunity against this entire group of diseases, actively by prophylactic injections of toxoid and passively by the administration of immune serums to actively ill patients.

AUTHOR'S SUMMARY.

*TETANUS TOXOID IN PROPHYLAXIS AGAINST TETANUS.* D. H. BERGY and S. ETRIS, *J. Infect. Dis.* **53**:331, 1933.

Experiments with tetanus toxoid on guinea-pigs showed that three doses of 1 cc. each bring about a high degree of protection. From the results obtained in guinea-pigs, it is believed that man may also be protected against tetanus by the administration of several doses of toxoid. This method of protection obviates the hypersensitization against horse serum that may occur from passive immunization with tetanus antitoxin.

AUTHORS' SUMMARY.

THE PURIFICATION OF DIPHTHERIA TOXOID. G. F. LEONARD and A. HOLM, *J. Infect. Dis.* **53**:376, 1933.

A method for the purification of toxoid on a large scale should meet the following requirements: easy manipulation, satisfactory yield, elimination of more than 50 per cent of the nonspecific protein, flocculation, sterilization by the Berkefeld filter, stability for at least one year, and a higher antigenic efficiency than the average crude toxoid. None of the methods tested has met all these requirements. Precipitation by ammonium sulphate with subsequent dialysis and precipitation by acetone and by alum gave the highest yields as measured by flocculation with antitoxin. The immunizing value of toxoids precipitated by alum was found to be far superior to that of the crude toxoids.

AUTHORS' SUMMARY.

## Tumors

HISTOLOGIC GRADING OF CARCINOMA OF THE BREAST. C. D. HAAGENSEN, *Am. J. Cancer* **19**:285, 1933.

In a series of 164 cases of carcinoma of the breast, which was exceptionally advantageous for a study of the relationship of histologic structure to prognosis because of the remarkably complete follow-up data and because of the superior quality and the large number of the histologic sections, a careful analysis has been made as to the prognostic significance of fifteen different histologic characteristics. Six of these characteristics have been found to have a probable relationship to the end-result of treatment. These significant characteristics are, in fact, similar to those which Hansemann originally proposed for the determination of the grade of anaplasia. The factors of fibrosis and lymphocytic infiltration, recently stressed in tumor grading, have been found to be without prognostic import. On the basis of these six significant histologic characteristics it would appear that carcinomas of the breast can be classified into three grades of malignancy, in which increasing grade of anaplasia parallels increasing grade of malignancy as evidenced by the tendency of the tumor to metastasize and kill the patient at an early date. It should be remembered that a prognosis based on this type of histologic evidence cannot claim mathematical accuracy. It should be regarded as an approximation and a rough one at that. Malignancy is a biologic phenomenon and does not lend itself to exact measurement in mathematical terms. Moreover, the extremes, that is, grades 1 and 3, should be given more weight than grade 2, the less definite middle class into which a great proportion of carcinomas of the breast fall. Knowledge gained from histologic grading should not be regarded as competing with clinical data bearing on prognosis, to which it is, of course, subordinate in importance. Histologic grading should rather be considered as a new and additional, a modest yet valuable increment to knowledge of the disease.

AUTHOR'S SUMMARY.

HISTOLOGICAL FACTORS IN THE PROGNOSIS OF MAMMARY CANCER. W. A. EVANS, JR., *Am. J. Cancer* **19**:328, 1933.

An analysis of some microscopic characteristics in regard to prognosis has been made in seventy-five operable cases of carcinoma of the breast treated by radical operation and short wavelength radiation. In considering the series as a whole, anaplasia (lack of differentiation) with respect to tubule formation, characteristics of the cytoplasm and irregularities of the cells indicates a shortened postoperative duration of life. The difference, however, is so slight that in the individual case anaplasia is of no practical importance in determining the outcome. The relative amount of fibrous tissue stroma is of no significance. A high degree of lymphocytic infiltration appears to be unfavorable. The addition of radiation therapy to surgical treatment does not appear to alter the factors on which a prognosis may be based.

AUTHOR'S CONCLUSIONS.

HISTOGENESIS OF EWING'S SARCOMA OF BONE. P. J. MELNICK, *Am. J. Cancer* **19:353**, 1933.

A consideration of the histogenesis of Ewing's sarcoma of bone leads to the following conclusions: It is not an endothelioma, because tumors derived from endothelium can only be identified by their possession of unmistakable vasoformative properties. It is not a reticulo-endothelial sarcoma, because it possesses few of the morphologic and none of the physiologic characteristics of the cells of the reticulo-endothelial system. It is not a form of myeloma, because the blood elements that are apparently a feature of some cases are probably part of the reactive process of periosteal new formation of bone and bone marrow in the periphery that is characteristic of this tumor. It is an undifferentiated round cell sarcoma. It probably originates from the undifferentiated embryonic mesenchymal cells in the connective tissue about the blood vessels in the haversian canals.

AUTHOR'S SUMMARY.

POLYMORPHOUS SARCOMA OF THE UTERUS. R. J. NEEDLES, *Am. J. Cancer* **19:364**, 1933.

Sarcoma of the uterus presents a varied cell picture and a certain degree of polymorphism. There is, however, a small group of tumors of the uterus with a cell picture so varied that they deserve to be termed "polymorphous sarcomas." Endotheliomas and giant cell tumors of the uterus show a similarity to certain cell groups found in polymorphous sarcoma. More thorough examination of different portions of some of these tumors might result in their being transferred to the polymorphous group. Three cases are reviewed which seem undoubtedly members of this group. A new case is reported, which makes a total of four cases to date.

FROM AUTHOR'S CONCLUSIONS.

THE CALCIUM, POTASSIUM, AND INORGANIC PHOSPHATE CONTENT OF THE BLOOD IN CANCER. H. JACKSON, JR., and F. H. L. TAYLOR, *Am. J. Cancer* **19:379**, 1933.

In thirty-one patients with malignant disease the blood calcium was within normal limits or above in approximately 50 per cent of the cases. The evidence indicates that the hypocalcemia existing in some cases of cancer is due to disturbed nutrition and not to cancer per se. An analysis of the thirty-one cases is presented with respect to the duration and localization of the tumor, the loss of body weight, the involvement of the bones and the gastro-intestinal tract and the age of the patient in relationship to the blood calcium. There were no changes in the level of the potassium or of the inorganic phosphate of the serum. Data are presented on the influence of roentgen therapy on some of the more important inorganic blood constituents. Intensive irradiation appears to be without effect on these substances in cases of cancer.

AUTHORS' SUMMARY.

TAR CANCER IN MICE ON DIETS SUPPLEMENTED WITH FRESH LIVER. A. F. WATSON, *Am. J. Cancer* **19:389**, 1933.

There is an increased carcinogenic response in tar-treated mice when the diet on which they are maintained is supplemented with fresh liver. The increased response is shown by the earlier appearance of the benign warts and by the larger numbers of animals that have warts and epitheliomas. On the other hand, the average interval of time between the first treatment with tar and the development of malignant growth is not reduced. If the administration of liver is discontinued after the appearance of the warts, there is no difference in the rate of development of malignancy. The results indicate that it is possible to modify the susceptibility of a tissue to the development of malignancy in response to the action of a carcinogenic agent. Under the experimental conditions adopted, this modification influences the rate of appearance and the numbers of warts; it also affects the

number of malignant tumors, but it does not accelerate the onset of malignancy. This suggests that the development of benign hyperplasia is conditioned, partly at any rate, by different factors.

AUTHOR'S SUMMARY.

INFECTIOUS PAPILLOMATOSIS OF RABBITS. R. E. SHOPE, *J. Exper. Med.* **58**:607, 1933.

A papilloma has been observed in the wild cottontail rabbit and has been found to be transmissible to both the wild and the domestic rabbit. The clinical and pathologic pictures of the condition have been described. It has been found that the causative agent is readily filtrable through Berkefeld but not regularly through Seitz filters, that it stores well in glycerol, that it is still active after heating to 67 C. for thirty minutes, but not after heating to 70 C., and that it exhibits a marked tropism for cutaneous epithelium. The activities and properties of the papilloma-producing agent warrant its classification as a filtrable virus. Rabbits carrying experimentally produced papillomas are partially or completely immune to reinfection and, furthermore, their serums partially or completely neutralize the causative virus. The disease is transmissible to domestic rabbits, producing in this species papillomas identical in appearance with those found in wild rabbits. However, the condition is not transmissible in series through domestic rabbits. The possible significance of this observation has been discussed. The virus of infectious papillomatosis is not related immunologically either to the virus of infectious fibroma or to that of infectious myxoma of rabbits.

AUTHOR'S SUMMARY.

MITOGENIC RADIATION BY CERVICAL CANCER. J. KLENITZKY, *Ztschr. f. Krebsforsch.* **39**:60, 1933.

Klenitzky describes an apparatus by which he was successful in detecting mitogenic radiation by cervical cancers in situ. The phenomenon was constantly present, but it occurred as well in certain noncancerous conditions—notably erosions—so that it cannot be used as a differential diagnostic measure. Nor is absence of the radiation in cancerous blood of diagnostic value, since this, too, may occur in other diseases; also, with squamous cell cancers, the radiation in the blood may persist. In a mouse with a transplanted adenocarcinoma, radiation in the blood returned after successful extirpation of the tumor and persisted until the death of the animal.

H. E. EGGERS.

CILIATED EPITHELIAL CYST OF THE LIVER. W. DULLIN, *Ztschr. f. Krebsforsch.* **39**:80, 1933.

In connection with this brief report on a case of ciliated epithelial cyst of the liver, Dullin states that it is the sixteenth case of the sort to be reported.

H. E. EGGERS.

SEASONABLE FLUCTUATIONS IN THE MORTALITY FROM CANCER. P. GEREB, *Ztschr. f. Krebsforsch.* **39**:104, 1933.

Known seasonal fluctuations in death rates are caused ordinarily by a complex of factors frequently defying definite evaluation. The incidence of certain infections and that of diseases due more or less directly to vitamin deficiencies reach their apex in the winter and spring; respiratory diseases and infantile spasmophilia are most frequent in the spring; intestinal infections, especially those of children, in summer; typhoid fever, in late summer and fall. In general, however, the maximum total death rate occurs in the first half of the year. Cancer is an interesting exception to this, as the mortality from it reaches the highest point in the last half of the year, standing in this respect in definite contrast to that from tuberculosis, with which the reverse is true. A similar periodic variation is shown in the rate of growth of transplanted tumors, and Gereb calls attention to the fact that this period of apparent greatest growth of tumors occurs at the time of an ample sufficiency of vitamins in the diet.

H. E. EGGERS.

MIXED TUMOR OF THE BREAST IN DOGS. K. NIEBERLE, *Ztschr. f. Krebsforsch.* **39**:113, 1933.

The mixed tumor of the breast appears to be the commonest one at that site in the dog just as in the human being it is the commonest one in the parotid gland. In the course of one year Nieberle saw fifty such tumors, while in the same period he saw only seven carcinomas and one adenofibroma of that organ. Almost without exception they were perfectly encapsulated and definitely benign. Like similar tumors in man, they sometimes showed wide variation in the morphologic elements. Glandular epithelium was quite constant, in the form of tubules or in that of simple or papillary cysts. Stratified squamous epithelium was more rare. Mucoïd connective tissue was fairly constant, and cartilage was frequent; occasionally they contained bone; another element seen with some frequency was a nest of reticular cells. In regard to the origin of such tumors, Nieberle dismisses absolutely the endothelial theory of Volkmann, as well as that of purely epithelial derivation. He regards them as resulting primarily from aberrant embryologic development, with the diversity of stroma being due to the effect, on undifferentiated mesenchyme, of the adjacent epithelial structures.

H. E. EGGERS.

NERVES IN TUMORS. M. N. MEISSEL, *Ztschr. f. Krebsforsch.* **39**:128, 1933.

Meissel used in this study of the nerve supply in malignant tumors those developing in tarred mice at sites remote from the areas of tarring. Both precancerous papillomas and squamous cell carcinomas were studied. In both there were abundant nerve fibers, reaching, in the former, to the keratinized cells, and originating in the nerve supply of the hair follicles; in the malignant tumors they appeared for the most part to be newly grown, and this growth could be traced back to nerve cords in the underlying connective tissue. Where nerve cords were compressed by the growing tumor they showed marked degeneration and active regeneration, similar to that seen after experimental trauma. Such regenerating and proliferating nerves penetrated through cancerous tissue, even reaching the epithelial pearls, and at times, in the form of fine filaments, establishing close contact with cancer cells. Some of them were of the morphologic type of the normal intra-epithelial nerves. Consequently, in these tumors there were remnants of nerves previously present, as well as such as were newly introduced through the processes of proliferation and regeneration.

H. E. EGGERS.

CANCER OF THE ANTRUM AND METAPLASIA. W. SCHÜTZ, *Ztschr. f. Krebsforsch.* **39**:152, 1933.

In a study of the mucosa of the antrum in fifty cases, Schütz could detect with certainty metaplasia in four, cylindric epithelium being altered to that of squamous cell or undifferentiated (basal cell) type. Morphologically, this appeared to be metaplasia of the type which Teutschlaender designated as prosoplastic. Metaplasia by dedifferentiation, either direct or indirect, did not appear to occur. The change was apparently accomplished by the basal cells. Schütz regards it as a precancerous process which in suitable subjects develops into malignant neoplastic growth.

H. E. EGGERS.

A PRIMARY CARCINOMA OF MECKEL'S DIVERTICULUM. K. FRANKE, *Ztschr. f. Krebsforsch.* **39**:206, 1933.

A man, 54 years of age, with the symptoms of acute ileus, was found to have, about 1.25 mm. above the ileocecal valve, a superficially ulcerated tumor from which there escaped slimy mucoïd material enough to fill the peritoneal cavity. Its diverticular character was evident grossly; histologically, its structure was that of a colloid cancer; there were, however, no metastases, and the course since its removal has been uneventful.

H. E. EGGERS.

# Society Transactions

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## PATHOLOGICAL SOCIETY OF EASTERN NEW YORK

ARTHUR W. WRIGHT, *Secretary*

*Regular Meeting, June 15, 1933*

R. J. LEBOWICH, *Presiding*

### FIBROMYOMA OF THE ASCENDING COLON. R. J. LEBOWICH.

At autopsy there was found in the ascending colon a solitary, roughly circular growth measuring 9 by 7.5 by 7 cm. This growth was freely movable, sharply circumscribed, completely encapsulated and covered over its superior surface by the markedly atrophied mucosa of the colon. It partly obstructed the lumen of the intestine. On section, the characteristic structure of a fibromyoma was revealed, free from degenerative or malignant changes.

Microscopic sections of the entire growth, stained with hematoxylin and erythrosin and with van Gieson's stain, showed equal amounts of fibrous tissue, focally hyalinized, and interlacing bundles of smooth muscle cells, uniform in size, shape and staining qualities.

### PRIMARY CARCINOMA OF THE LUNG. G. H. KLINCK, JR., and V. C. JACOBSEN.

CASE 1.—A white man, 59 years of age, was admitted to the psychiatric service because of moodiness and morbid ideas. Six weeks previously hoarseness had developed, followed by pain in the chest and abdomen. He gradually became stuporous, began to have difficulty in swallowing and died two weeks later.

At autopsy, the left lung weighed 450 Gm. There was a mass of lymph nodes, 8 cm. in diameter, at the hilus. These showed neoplastic invasion. In the bronchus there was a nodular tumor which greatly narrowed the lumen and spread into the adjacent pulmonary tissue. No other mass was found. The nodular tumor was found to be continuous with a neoplastic mass, 2.5 by 1 by 1 cm., in the pulmonary substance. The right lung was not involved. The liver, kidneys and suprarenal glands showed extensive metastases.

Microscopically, the tumor in the left bronchus was a carcinoma infiltrating beneath and eroding through the bronchial mucosa. There was a circumscribed infiltration of the pulmonary substance in this region. The tumor was composed of oat-shaped epithelial cells, which in places maintained a more or less parallel arrangement. There were numerous mitoses and many large areas of necrosis. Secondary growths which were identical histologically with the parent tumor were found in the kidneys, suprarenal glands and brain.

CASE 2.—A white man, 78 years of age, noticed a nodule in the lower portion of the right side of his neck. Biopsy showed a spindle cell carcinoma. The primary tumor could not be found. He became comatose and died three days later.

At necropsy, the left lung weighed 810 Gm. and contained a large, firm tumor in the superior half of the upper lobe. The bronchus showed an elevated, firm, white, nodular growth 2 or 3 cm. from the bifurcation of the trachea. From this point the growth spread out in a rather fan-shaped manner over an area 4 by 6 cm. The lower lobe of the left lung was free from nodules. The right lung contained numerous small, hard nodules from 1 to 3 cm. in diameter. The liver, both kidneys, the left suprarenal gland and the mesenteric, retroperitoneal and mediastinal lymph nodes contained metastases. In the bladder numerous diverticula

were present, the largest measuring about 3 cm. in diameter. In this large pouch was found a firm, slightly fungating mass 3 cm. in diameter, occupying almost the entire thickness of the wall. No calculi were present.

Microscopic examination of the left lung showed a primary medullary carcinoma arising in the left bronchus. It had invaded widely, and groups of tumor cells were found in many blood and lymph vessels. The cells in places suggested origin either from glandular or from ciliated epithelium. Necrosis was present in the larger masses and mitoses were frequent. The hilar lymph nodes were heavily infiltrated with tumor. Metastases were present in the liver, suprarenal glands, prostate gland and vertebrae and in a diverticulum in the bladder.

The object in presenting these cases is twofold: It is first to draw attention to the fact that, in the state of New York at least, the incidence of cancer of the lung is increasing and, second, to demonstrate two well recognized types of primary bronchial carcinoma, the type in which small oat-shaped cells predominate and the type in which columnar or spindle-shaped cells are characteristic and which arises definitely in a glandular or a ciliated columnar cell. An unusual feature in the latter case was the metastasis to the diverticulum in the bladder, an origin in the diverticulum being ruled out. The tendency of pulmonary cancer to metastasize to the brain is worthy of emphasis.

#### CORONARY OCCLUSION, THROMBOSIS IN THE LEFT VENTRICLE, ILIAC EMBOLISM AND GANGRENE OF THE LOWER EXTREMITIES. J. J. CLEMMER.

A white man, aged 57, while working as a night watchman, was seized with severe epigastric pain, followed by prostration. Five days later he complained of pain in his right leg and two days after that clinical signs of gangrene were observed in the right foot. The gangrene progressed to the leg and in a few days the left lower extremity became similarly involved. The gangrene of both feet and legs progressed and death occurred, hastened by terminal pneumonia, six weeks after the onset of the symptoms.

Necropsy revealed an enlarged heart, marked coronary sclerosis and recent occlusion of the descending branch of the left coronary artery. The myocardium in this region was partially replaced by young, vascular, fibroblastic tissue. Portions of the remaining myocardium showed retrograde changes. Mural or valvular thrombi were not present.

The aorta was sclerotic. A laminated fibrinous mass protruded from the right iliac artery and impinged on the opening of the left. Both legs and feet were gangrenous, the right more so than the left. Several relatively recent infarcts were found in the spleen. A single hemorrhagic infarct was present in the right kidney. There was terminal acute bronchopneumonia.

It is believed that the acute onset was due to the coronary occlusion. Soon a mural thrombus formed in the left ventricle. As the myocardium partially recovered it forced the thrombus into the systemic circulation. Small infarcts of the spleen and kidney resulted, but the main mass lodged in the right iliac artery. This protruding embolic mass grew by accretion until it produced obstruction of the left iliac artery. Gangrene of the lower extremities developed as a result of the impaired circulation.

#### OSTEITIS FIBROSA ASSOCIATED WITH PARATHYROID ADENOMA. ELLIS KELLERT.

A man, 37 years of age, had symptoms of urinary disturbance for ten years. He frequently passed minute calculi and many pus cells in the urine. More recently there were loss of weight and signs of renal insufficiency. Roentgenograms of the kidneys did not reveal calculi or enlargement of the organs. Owing to pain in the bones, these were roentgenographed and a generalized osteitis fibrosa was observed. The neck appeared normal on inspection and palpation. Secondary anemia was present, and there was definite retention of nonprotein nitrogen. The calcium in 100 cc. of blood amounted to 15 mg. After a period of epigastric distress and vomiting, death took place in coma.

Necropsy was limited to examination of the neck and kidneys. There was no tumor of the stomach, intestine or suprarenal glands. The right kidney was one-third larger than the left. Each kidney contained abundant renal substance, which was markedly congested and studded throughout with numerous small and large calcific particles. In the cortices were many small cysts filled with yellowish fluid. The pelves contained small calculi, and their mucous surfaces were covered with puriform material. Microscopic examination showed many sclerosed glomeruli, foci of lymphocytic infiltration, castlike bodies in the tubules, marked fibrosis and calcification of the pyramids. Deposits of calcium were observed in the interstitial tissue and within the remains of tubules.

The thyroid gland appeared normal. Situated on the posterior surface of the lower pole of the right lobe was a yellowish, globular, firm, encapsulated nodule measuring 3.5 by 2.5 by 2.5 cm. The capsule was thick, fibrous, and partly calcified



Photograph of thyroid gland showing large parathyroid adenoma on posterior surface. Note enlarged parathyroid body just above the adenoma.

and had no direct connection with the thyroid substance. When the nodule was sectioned, the cut surface appeared pale brown, soft and friable, and there was a recent hemorrhage in the center. The parenchyma tended to split on handling. Two other parathyroid glands were identified, and these were distinctly enlarged. Microscopic examination of the tumor showed abundant but normal-appearing parathyroid gland tissue. The cells were sharply defined. Many of the nuclei were pyknotic. No mitoses were observed.

#### CARCINOSARCOMA OF THE BREAST. J. SCHLEIFSTEIN.

Tumors of the breast containing carcinoma as well as sarcoma are rare. The specimen now described appears to contain both carcinoma and sarcoma.

An unmarried woman, past middle age, complained of a lump of one year's duration in the lower part of the right breast which a month previously began to grow rapidly and became painful. Six months previously she thought that it was



becoming smaller and hoped that it would entirely disappear. The breast was removed under local anesthesia.

The specimen consisted of approximately the whole right breast, measured about 8 by 6 by 6 cm. and was covered with skin. A diffuse tumor with localized nodulations was felt. On section, most of the breast appeared to be invaded by new growth. Areas of hemorrhage and necrosis could be seen, as well as one large cavity about the size of a plum, filled with débris and necrotic material.

Sections from one part of the breast showed typical alveolar carcinoma with scanty stroma. However, those taken from the advancing edge of the tumor presented an entirely different picture: A diffuse sarcomatous growth consisting of large spindle cells with hyperchromatic nuclei containing numerous mitotic figures, as well as giant cells with bizarre mitoses and extremely large nuclei. Well formed carcinomatous alveoli seemed to be embedded in the sarcomatous stroma. In some areas, particularly where the sarcoma grew, hemorrhage and necrosis were seen. Sections stained with Mallory's phosphotungstic acid hematoxylin showed what appeared to be fibrils. It is unfortunate that the lymph nodes of the axillary region were not submitted for examination.

#### FIBRO-ADENOMA OF THE BREAST CONTAINING CARCINOMA AND CARTILAGE. J. SCHLEIFSTEIN.

Cheatle, in his vast experience with tumors of the breast, has seen only two neoplasms of this organ containing cartilage. The occurrence of this type of tumor in man appears to be unusual, although it is frequent in the dog. A case that I observed presents features of interest to both the clinician and the pathologist.

A married woman, aged 57, complained of a lump of three months' duration in the left breast. There was no pain or tenderness. The nipple was not retracted or deformed. A tumor about the size of a peach, firm and circumscribed, was felt. No glands were palpable in the axilla. At operation what appeared to be an encapsulated fibro-adenoma was easily enucleated.

A portion of the tumor, about 4 by 3 by 3 cm., was received. The tissue was found to be rather firm, grayish white and fibrous with occasional chalklike specks embedded in it. Toward the center there was a minute, grayish-white, glistening nodule.

The microscopic examination showed an anaplastic, malignant growth, having the characteristics of an epithelial tumor, invading and infiltrating the fibro-adenoma. Hyperchromatic nuclei, numerous mitotic figures and some giant cells were found. Toward the center of the section a small nodule of cartilage was observed. Whether the carcinoma originated elsewhere in the breast and invaded the fibro-adenoma or was primary in the fibro-adenoma is difficult to determine since the whole breast was not available for study.

#### PERSISTENT ENTERIC MUCOSA AT THE UMBILICUS. ARTHUR W. WRIGHT.

A 13 year old white boy complained for one year of a small, moist, slightly painful, vascular tumor of the umbilicus. A physician removed the growth, which consisted of a globular mass of firm, skin-covered connective tissue, on the surface of which was the umbilical depression. In this depression there was a circular, congested, moist, yellowish-pink, slightly raised lesion which did not appear to be a tumor or an ulcer but which was distinctly abnormal. Microscopically, the mass proved to be evaginated intestinal mucous membrane which was congested and slightly inflamed. There was no evidence of malignant growth or ulceration. The lesion, clearly congenital, undoubtedly resulted from abnormal isolation of an island of enteric mucosa from the primitive vitelline duct which, during early embryonic life, extends out into the umbilical cord.

## BUFFALO PATHOLOGICAL SOCIETY

*Regular Meeting, Oct. 29, 1933*KORNEL TERPLAN, *President, in the Chair*

## HISTOLOGIC DIAGNOSIS OF PREGNANCY FROM PLACENTAL FRAGMENTS. C. S. RYERSON and S. SANES.

This article will appear in the ARCHIVES.

## CONGENITAL HEART DISEASE: INTERVENTRICULAR SEPTAL DEFECT, DEXTROPOSITION OF AORTA, AND DILATATION OF PULMONARY ARTERY. RAYMOND S. ROSEDALE.

The anomaly consisted of an unusual combination of dextroversio cordis, hypertrophy of the pulmonary artery, defect of the interventricular subaortic septum and dextroposition of a relatively small aorta. This was found in a 10 year old boy who since birth had been persistently troubled with dyspnea and cyanosis after exercise or emotional disturbance.

The foramen ovale was obliquely patent and measured 0.5 by 1 cm. The interventricular septal defect measured 2.5 by 3 cm. The diameters of the pulmonary artery and ascending aorta were as three to one. The large size of the closed ductus arteriosus suggested that it had been patent for some time after birth. There was hypertrophy of the musculature of both the left and right ventricles. The great branches of the aortic arch were anomalous, falling somewhat into the fifth class of anomalous great arteries as outlined by Piersol.

It was thought that the blood in the left ventricle had been partially shunted to the right ventricle. This was considered to have been due somewhat to a large muscle bundle that partially obstructed the aortic outlet. The fibrous portion of the aortic pulmonary septum was found to end blindly in this muscle bundle.

The normal equal division of the truncus and bulbus arteriosus had not taken place, for the remainder of the aorta which is not concerned with the septum trunci was of the same caliber as the ascending portion. The increase in size of the pulmonary artery and bulb was interpreted as hypertrophy and dilatation, postnatal in development.

It is suggested that the subaortic interventricular septal defect was probably due to the small size of the septum trunci and its failure to fuse with the posterior endocardial cushion.

The immediate cause of death was assumed to have been a general dilatation with coronary thrombosis; intimal thickening with plaques of adherent thrombi was noted in all the sections of the coronary and its branches.

A review of the literature revealed only four similar cases. The first was reported by Eisenmenger in 1897. The others were reported by M. E. Abbott and co-authors, who have referred to the combination of interventricular septal defect with dextroposition of the aorta and dilatation of the pulmonary artery as the "Eisenmenger complex."

## ACUTE PNEUMOCOCCIC ENDOCARDITIS IN EARLY INFANCY. S. SANES and F. E. KENNY.

Of reported cases of acute bacterial endocarditis in infancy, only six can be accepted as adequately verified anatomically and bacteriologically (Blum, Dean, Dible, Terplan and associates, d'Evart, Brandes). In twenty-six other cases bacteriologic proof was not established; anatomic changes and occasionally embolic phenomena were noted, however.

CASE.—A white boy, aged 13 days, was admitted to the Children's Hospital because of cough, cyanosis and anorexia of three days' duration. The temperature was 103 F.; the pulse rate, 80. The nose was filled with purulent exudate; the

throat was red; there were diminished breath sounds and râles in both lungs. The heart was not enlarged; no thrill or murmur was elicited; the rate was regular, and the sounds were of good tone. The white blood cell count was 18,700, with 60 per cent polymorphonuclears and 40 per cent lymphocytes. On the ninth day a subcutaneous abscess appeared in the lumbar region; pneumococci were obtained in smears and cultures. Death occurred on the twenty-eighth day of life.

Autopsy, three hours after death, showed: confluent lobular pneumonia of the right upper and lower lobes, with small abscesses; a large abscess in the left psoas muscle extending into the subcutaneous tissue at the level of the third and fourth lumbar vertebrae; vegetative endocarditis of the ventricular surface of the tricuspid valve and of the posterior wall of the right ventricle beneath the attachment of the valve and of the conus pulmonaris; slight dilatation of the right ventricle, and congestion of the liver, spleen and kidneys. Smears and cultures from the vegetations and the lung revealed pneumococci.

Histologically, the right ventricle showed parietal endocarditis with fibrinous deposits containing many degenerated leukocytes and pneumococci. The tricuspid valve showed small vegetations directly beneath its attachment, with marked necrosis at its base and leukocytic infiltration. Pneumococci were found scattered throughout the vegetations as well as at the base of the valve. There were also pneumonic changes with fibrinous and leukocytic exudate and congestion of capillaries.

PRIMARY TUBERCULOUS FOCUS WITH EXTENSIVE CASEOUS PLEURITIS WITHOUT COMPLEX CHANGES IN THE REGIONAL LYMPH NODES. K. TERPLAN.

This case is presented because the anatomic findings showed an entirely unusual type of spread from a cheesy Ghon focus.

A white American woman, 23 years of age, was delivered spontaneously of a baby at full term, June 5, 1932. June 18, chills and fever made their appearance. These continued to occur for three days; then the patient was forced back to bed with headache, nausea and emesis. She was admitted to the hospital, June 27. The uterus was in position; there were no palpable evidences of extra-uterine infection. July 8, rigidity of the neck was evident. The patient died four days later.

The family history and past history could not be satisfactorily obtained.

A complete postmortem examination was made forty-eight hours after death. The body was emaciated, the skeletal system slender, the subcutaneous fat almost entirely absent.

The left lung was completely adherent to the parietal pleura, especially that on the diaphragm, mediastinum and pericardium. The right lung was free, with compensatory emphysema. The organs of the neck, thorax and abdomen were removed in toto; the thoracic duct was carefully dissected. In the removal of the left parietal pleura, a few small cheesy structures were revealed in the intercostal soft tissue above the anterior and lateral surfaces of the left lower lobe. In the left pleural cavity the mediastinal and diaphragmatic pleura was involved by marked cheesy infiltrations; the shape of the left lower lobe was changed, its base being distinctly contracted, measuring only 12 cm. in frontal diameter and 4.5 cm. in sagittal diameter. This was due to inversion of the basal margin, especially on the posterior side; the entire parietal pleura was thickened and pinkish gray and contained many tubercles; some of these invaded the diaphragm.

About 1 cm. above the base of the lung near the interlobar surface was a pea-sized caseous focus, from 0.5 to 0.6 cm. in diameter, immediately beneath the pleura. The pleura above this focus was firm, also inverted, and showed on cross-section caseous fibrosis. Caseous changes were also present on the external surface of the pericardium in connection with the caseous tuberculosis of the mediastinum on the left side. In the left upper and lower lobes were many pinhead-sized tubercles; those in the apex were somewhat larger and somewhat closer to each other.

There were no gross tuberculous lesions in the bronchomediastinal lymph nodes on the left side.

The lobes of the right lung showed numerous pinhead-sized tubercles. The lymph nodes on the right side were small and anthracotic. Only the lymph nodes in the left venous angle were grayish white, suggesting tuberculosis. All the lobes were dissected in very thin sheets. X-ray pictures were taken from the undissected lung as well as from the sheets of both lungs.

Older tuberculous lesions were not present in the intestine or in the mesenteric lymph nodes. Careful dissection of all organs, especially all lymph nodes, did not reveal a primary extrapulmonary tuberculous infection. A few of the left broncho-mediastinal nodes showed, however, microscopic tuberculosis with epithelioid cell tubercles, but without caseation. These changes were apparently secondary to the marked caseous pleuritis on the left side. The primary focus showed complete caseation in process of partial fibrous organization. Typical miliary tubercles were present in the leptomeninges, liver and spleen, and tuberculous infiltrates in the kidneys. The uterus was completely involuted and showed only small placental remnants without tuberculosis.

Anatomic dissection with thorough roentgen examination indicated that the caseous tuberculosis of the left pleural cavity had spread from a late primary caseous subpleural fibrous focus. The lymph nodes regional to the left lower lobe did not show caseous changes; in other words, no primary complex was formed. The tuberculous changes in the lymph nodes were microscopic and could be explained easily by the marked caseous pleuritis on the left side.

The entire picture can hardly be explained in any way other than by assuming an overwhelming primary infection, possibly acquired during pregnancy. Why the pleural cavity at once was infected to such a large extent as to produce diffuse caseous pleuritis, and why a lymphogenous spread to the lymph nodes regional to the focus did not occur, is difficult to state. It is known that the pleura above primary foci may be involved locally; very often even adhesions form. But such a pleural involvement remains restricted to the surface of the focus, while the lymph nodes draining the focus become involved very soon.

As the postmortem examination was complete and all organs were investigated for primary tuberculous lesions with negative results, it seems justified to consider the focus in the left lower lobe as the primary one in this case. Apparently, the overwhelming infection of the pleural cavity prevented the so-called complex changes in the lymph nodes regional to the focus.

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*Joint Meeting with the Buffalo Academy of Medicine, Nov. 29, 1933*

MARGARET WARWICK, *President, in the Chair*

#### ANATOMIC STUDIES OF PRIMARY AND POSTPRIMARY PULMONARY TUBERCULOSIS IN WHITE CHILDREN AND ADULTS. K. TERPLAN.

Much of the present knowledge of primary human tuberculosis is based on careful gross pathologic work (Parrot, Kuss, E. Albrecht, Ghon, H. Albrecht and Opie). In progressive pulmonary tuberculosis in adults, the anatomic picture tends to be complicated; often it seems difficult to trace the changes back to the first lesion. It is generally believed that the disease usually develops in connection with a so-called reinfection. There is some controversy as to the manner of this reinfection; that is, as to whether it is a reinfection from outside the body while the first lesions are healing or are still active, or whether it is a so-called endogenous reinfection or exacerbation originating in the primary focal lesion or in the regional lymph nodes. This question ought to be studied individually in each case. The structural lesions in the lungs of adults who have died from progressive chronic pulmonary tuberculosis are often too extensive to serve properly as material for pathogenic studies. On the other hand, in examining cases in which death was not due to tuberculosis, many pathologists, satisfied with finding one typical calcified tuberculous complex, have assumed that this complex was the only lesion. The x-ray

picture has helped in detecting calcified foci, and one usually has taken for granted that round shadows of calcification represented tuberculous foci.

As far as early changes, especially in so-called reinfection, are concerned, incidental findings may sometimes be of great value. Because the early lesions are usually less extensive it is easier to discover the paths along which they developed.

The study of the pathogenesis of tuberculosis cannot be thorough enough. It not only has to include minute dissection and taking of roentgenograms, but also a complete histologic examination of all focal lesions found by roentgen examination and dissection.

In the present study I have attempted merely to register and analyze all findings of a tuberculous nature without trying to correlate them with present theories derived from experiments on animals which are so readily used to explain in general terms of immunity the individually different lesions of human tuberculosis.

From the data contributed in the past fifteen years a few more or less dogmatic statements have been made. For example: All children in cities acquire their first tuberculous lesion before they reach adult life. Tuberculosis in adults acts *altogether differently from that in children; it usually starts in the apex; it is acquired from without; the tributary lymph nodes are not involved.* When miliary tuberculosis occurs in adults it is only because they have escaped childhood infection. Small foci give scant resistance; marked focal lesions give marked resistance.

It is customary to group all tuberculous lesions into childhood and adult types. This predicates that tuberculosis is always first acquired in childhood, and that tuberculosis in adults usually does not show the striking lesions of first infection, with a primary focus and involvement of regional lymph nodes. I prefer the terms "primary tuberculosis" and "postprimary tuberculosis." The latter is wide enough to cover the different ways of so-called reinfection.

It is stated that adults react like reinfected animals in the allergic stage. Still one does not know why these reactions in man vary so markedly, sometimes remaining localized in fulfilment of the experimental postulates, and sometimes spreading not only along the bronchi, but also to lymph nodes, like a primary lesion, and through blood vessels. Medical examination does not prove that with a similar exposure children of the same family are apt to acquire progressive tuberculosis or so-called reinfection. Tuberculosis in man appears much more complicated in its origin and course than that produced by well graded experiments on animals. Spontaneous tuberculosis in animals comes surely closer to being the counterpart of human tuberculosis.

Modern advances especially in the treatment and prevention of tuberculosis have a distinct bearing on the apparently decreasing incidence of tuberculous lesions in childhood in the white race. Although the total number of children examined in this study is small (271 from 1 month to 6 years of age, and 50 from 7 to 18 years of age) the figures show that primary tuberculosis does not always occur during childhood, but apparently occurs rather in the early years of adult life. In general, the figures indicating the incidence of focal tuberculous lesions up to the eighteenth year vary between 20 and 25 per cent. They are apparently in line with the figures on positive reactions to tuberculin tests as published by pediatricians in Massachusetts, Minnesota, Wisconsin and Cattaraugus County, New York, and from different schools in Buffalo. The number of children giving positive reactions gives only the lowest number of the once infected children, as it is likely (proved by my experience in two instances) that with anatomic healing of small focal lesions, the reaction to tuberculin becomes negative again. With exception of Opie's work in St. Louis and Philadelphia, no systematic postmortem examinations have been made in this country with a view to studying the incidence of primary focal lesions in children and young adults. Apparently it has been taken for granted from the figures of Kuss, Ghon, Aschoff and Opie that primary foci are acquired by all children before they reach adult life.

The total number of persons between the twentieth and fortieth years of life examined postmortem for this study is again small (total number 28) and cannot be used as a basis for statistical study. However, the majority of them (from

67 to 75 per cent) showed lesions of primary tuberculosis. After the fortieth year of life the person who revealed no such lesion was exceptional. I encountered primary focal lesions with caseous and caseofibrous complexes in about eight persons between the fourth and the eighth decade of life. These lesions acted similarly to first lesions seen in childhood. The development of progressive tuberculosis is not, except possibly in infancy and early childhood, predominantly determined by the age of the patient. In the study of focal lesions histologic examination of older calcified and ossified foci affords no means of distinguishing absolutely between primary and postprimary tuberculous infection. The older the lesions the more exactly do they resemble each other, each exhibiting remnants of so-called caseous pneumonia which can be demonstrated in a calcified stage by stains for elastic tissue. The mesenchymal reaction surrounding localized lesions may or may not differ in primary and postprimary foci. Puhl's criteria can be used only in the small number of cases in which the postprimary lesions are progressing or are in the early stages of healing. Contrary to commonly accepted teaching, the findings which I report indicate that postprimary infection may produce complex changes identical with those seen in primary tuberculosis. Progressive postprimary lesions often tend to be accompanied by changes in regional lymph nodes. These changes are in general microscopic. In older persons, however, they may be just as active and extensive as are those in many cases of primary infection. Some instances of this active, extensive development may represent the so-called lymphendogenous reinfection.

The presence of ossified healed lesions on one side and of older ossified complexes on the other may be explained by a development of multiple primary lesions simultaneously with a one-sided spread, by a reinfection that occurred many years previously, or by a secondary bronchiogenic spread from the first lesion while it was still active. The last possibility appears to be substantiated in cases in which one finds, closely surrounding an old primary lesion, fibrous tubercles and recent caseous bronchiogenic spread.

In proved cases of reinfection apparently exogenous in origin, the site of lesion is most often in the upper half of an upper lobe, from 2 to 4 fingerbreadths below the apex. The summit or apex is only occasionally primarily involved, and the lower part of the upper lobe or the lower lobe, less frequently. With this distribution of secondary lesions, the clinical designation "infraclavicular tuberculous infiltrate" becomes more satisfactory than "tuberculous apical infiltrate."

To summarize: From the postmortem material examined in the last three years at the Buffalo General Hospital and the Children's Hospital it seems that the first tuberculous infection commonly takes place in the first three decades of life, especially from the age of puberty to that of from 20 to 30 years, and only occasionally later. Postprimary lesions may produce so-called complex changes in lymph nodes similar to those of primary infection.

How far multiple foci prevent lymphatic spread is a question that cannot be appropriately or satisfactorily discussed from the point of view of these studies dealing, as they do, chiefly with older or healed lesions. Although the law of Parrot still stands well founded as a general working principle, it must not be considered an absolute, inflexible dogma. As I have shown, primary focal lesions may remain entirely restricted in the lung, without changes in the regional lymph nodes. The incidence of focal lesions without complex changes cannot be accurately expressed, because many such lesions are probably overlooked unless a thorough search is made for them.

In a certain number of cases in adults and even in children marked progressive involvement of the lungs is not accompanied by acute miliary tuberculosis. A subacute hematogenous spread occurs. Tuberculous meningitis, however, may constitute the dénouement of the disease.

The practicability of adhering to a rigid classification of tuberculosis based on experimental findings becomes, in the light of the multiform lesions and varied courses in individual human beings, an open question.

## Book Reviews

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**De Venarum Ostiolis 1603 of Hieronymus Fabricius of Aquapendente (1533?-1619).** A facsimile edition, with introduction, translation and notes, by Kenneth J. Franklin, D.M., Tutor and Lecturer in Physiology of Oriel College and University, Demonstrator of Pharmacology, Oxford. Price, \$3. Pp. 104, with 7 figures and 8 plates. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

This book is published by the aid of a grant from the History of Science Society, which has received an appropriation from the Carnegie Corporation of New York to enable it "to publish, or assist in publishing, important contributions to knowledge." In addition to the facsimile reproduction and translation of "De Venarum Ostiolis," published in 1603, the book contains a biographic note on Fabricius, a section on the early history of work on the venous valves, a short account of the anatomic theater at Padua and a bibliographic note, for which the dedicatee, Prof. J. F. Fulton, is chiefly responsible concerning the first edition. Besides reproductions of the original plates and the frontispiece showing Fabricius as portrayed in 1630, the illustrations include the following: the statue of Fabricius in Aquapendente; the earliest drawings of a venous valve (Alberti, 1585), the theater of Fabricius, with the plan and elevation, and a reproduction of a general title page of his "Opera anatomica," published in 1625. The book is printed with special care on Patten rag paper. The publisher is worthy of commendation for publishing at this time a book of historic interest in such attractive form.

In his preface Dr. Franklin points out that "the discovery of the circulation by William Harvey was in large measure due to his correct estimate of the function of the venous valves, and about these he had heard while studying under Hieronymus Fabricius at Padua from 1600 to 1602." He then quotes the following account by Robert Boyle of a conversation with Harvey: "And I remember that when I asked our famous Harvey, in the only Discourse I had with him (which was but a while before he dyed), What were the things that induc'd him to think of a Circulation of the Blood? He answer'd me, that when he took notice that the Valves in the Veins of so many several Parts of the body, were so Plac'd that they gave free passage to the Blood Towards the Heart, but oppos'd the passage of the Venal Blood the Contrary way: He was invited to imagine, that so Provident a Cause as Nature had not so Plac'd so many Valves without Design: and no Design seem'd more probable, than That, since the Blood could not well, because of the interposing Valves, be Sent by the Veins to the Limbs; it should be Sent through the Arteries, and Return through the Veins, whose Valves did not oppose its course that way." Hence one has in readily accessible and appropriate form the original and the translation of a book of fundamental importance in the history of the discovery of the circulation of the blood.

**Metabolic Diseases and Their Treatment.** By Dr. Erich Grafe, Professor of Medicine of the Clinic of Medicine and Neurology at the University of Würzburg, Germany. Translated by Margaret Galt Boise under the supervision of Eugene F. DuBois, M.D., Medical Director, Russell Sage Institute of Pathology, and Professor of Medicine, Cornell University Medical College, New York, and Henry B. Richardson, M.D., Associate Professor of Medicine, Cornell University Medical College, New York. Cloth. Price, \$6.50, net. Pp. 551, with 37 engravings. Philadelphia: Lea & Febiger, 1933.

The German edition of Professor Grafe's book was published in 1931. It was soon recognized as a comprehensive and conservative presentation of the modern German views on the diseases of metabolism. Grafe is particularly well fitted to

discuss the scientific and practical aspects of this subject. Trained in the school of Rubner, he has made original and important contributions in the fields of respiratory and intermediary metabolism. At the same time he has been advancing in clinical medicine and has attained one of the most important professorships in Germany. He is one of the few leaders in his country who have kept in touch with the American literature. His book covered the subject so well and filled such a large gap in the modern literature that Lea & Febiger arranged for a translation, with extensive revisions by Grafe which brought the material up to 1933.

The translation has evidently been made with considerable care and seems to preserve as closely as possible the original ideas of the author. The text still contains references to many German proprietary medicines with which physicians in this country are unfamiliar, but apparently the author has cut out about half of those which appeared in the original edition. One must remember that there is no Council on Pharmacy and Chemistry in Germany and that thousands of proprietary medicines are used throughout the Continent without adequate supervision. The Continental tendency toward inaccuracies in references was evident in the original; these inaccuracies have been partly corrected though not entirely eliminated in the translation. There are some typographical errors; for example, in table 22, page 193, 1.0 salyrgan appears as 1 mg. salyrgan. Both are wrong; the dose evidently should have been 1 cc. of a 10 per cent solution of salyrgan.

On the whole, the book is well written, and makes easy reading for one who is interested in the diseases of metabolism. For the teacher and research worker it is a valuable source book. The chapters on obesity and malnutrition are particularly good. There is an excellent discussion of the theories and treatment of gout by an observer who has had unusual opportunities for studying the disease at first hand. The subject of diabetes is well handled, and there are brief but adequate chapters on the rarer diseases of metabolism and the vitamin deficiencies. There are no special sections devoted to basal metabolism or to diseases of the thyroid gland, but references to these are scattered throughout the volume. In general, the field of metabolism is covered in a critical and conservative manner.



## Books Received

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BAC. PARATYPHOSUS B BELYST VED UNDERSØKELSER OVER PARATYFUS-B-STAMMER FRA VEST-NORGE OG OVER SYGDOMMENS OPTREDEN SAMMESTEDS. T. M. Vogelsang. Pp. 235, with 9 figures. Bergen: J. W. Edes Boktrykkeri A/S, 1933.

This monograph is based on the study of strains of paratyphoid B obtained from patients with paratyphoid fever in the western part of Norway. Paratyphoid bacillus B is different from the bacteria of food poisoning and causes a typhoid-like disease, paratyphoid B fever. This disease is spread from person to person directly or indirectly and appears to be quite as specific as typhoid fever. Other sources of infection have not been demonstrated definitively.

HOUSING CONDITIONS AND RESPIRATORY DISEASE: MORBIDITY IN A POOR-CLASS QUARTER AND IN A REHOUSING AREA IN GLASGOW. C. M. Smith. Medical Research Council, Special Report Series, No. 192. Price, 9d. Pp. 36. London: His Majesty's Stationery Office, 1934.

AIDS TO PATHOLOGICAL TECHNIQUE. David H. Haler, M.B., B.S. (Hons.) Lond., Pathologist, Infants Hospital, Battersea General Hospital, etc. Price, \$1.50. Pp. 187. Baltimore: William Wood & Company, 1933.

This booklet does not deal with the examination of tissues. The methods described are mainly bacteriologic, hematologic, cytologic and biochemical—"one example of each method found most valuable."

DIE SPEZIFIZITÄT DER SEROLOGISCHEN REAKTIONEN. K. Landsteiner, the Rockefeller Institute for Medical Research, New York. Price, 8.80 marks. Pp. 123, with 22 tables and 1 illustration. Berlin: Julius Springer, 1933.

DISSECTING ANEURYSMS. T. Shennan. Medical Research Council, Special Report Series, No. 193. Price, 2s. 6d. Pp. 138, with 13 figures. London: His Majesty's Stationery Office, 1934.

## COMBINED SYPHILITIC AND RHEUMATIC DISEASE OF THE AORTIC VALVE

REPORT OF THREE CASES

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Authentic cases of combined syphilitic and rheumatic disease of the aortic valve are extremely rare. In a thorough investigation of the last six thousand, five hundred postmortem examinations performed at the Mount Sinai Hospital during the past twenty years only two acceptable cases could be found. A search of the literature failed to disclose any similar observations except possibly Stolkind's<sup>1</sup> case of syphilitic aortic insufficiency with mitral stenosis; in that case the absence of a microscopic description does not permit us to exclude the possibility of coexistent rheumatic disease in the aortic valve.

Combinations of rheumatic disease of the heart with syphilis of the aorta found at necropsy are, however, less rare. Cabot<sup>2</sup> (1926) stated that "rheumatic valve lesions rarely accompany syphilitic aortitis." Reference to the table indicates the various combinations of these diseases reported in the literature together with seven cases investigated by us. The syphilitic process was entirely supravulvar in four of the cases, while commissural syphilis was present in the remainder; the three latter cases are reported in detail.

Buday<sup>3</sup> and Reid<sup>4</sup> referred statistically to the association of rheumatic with syphilitic disease of the heart. The former enumerated ten cases of combined "syphilitic endocarditis" and "endocarditis chronica fibrosa" among approximately eight hundred specimens of endocardial

From the laboratories of the Mount Sinai Hospital.

This study was aided by a grant from the Lucius N. Littauer Foundation and by a grant made to the Emanuel Libman Fellowship Fund in memory of Theresa Backer.

1. Stolkind, E. J.: *Brit. J. Child. Dis.* **17**:126, 1920.

2. Cabot, R. C.: *Facts on the Heart*, Philadelphia, W. B. Saunders Company, 1926, p. 336.

3. Buday, L.: *Frankfurt. Ztschr. f. Path.* **38**:450, 1929.

4. Reid, W. D.: *Am. Heart J.* **6**:91, 1930.

disease. The latter stated that of seventy-eight cases of cardiovascular syphilis, two were definitely associated with rheumatic disease of the heart. In the absence of protocols it is impossible to evaluate these cases, particularly with reference to combined rheumatic and syphilitic disease of the aortic valve.

The following cases illustrative of combined rheumatic and syphilitic disease of the aortic valve are reported.

REPORT OF CASES

CASE 1.—A Puerto Rican driver, aged 27, was admitted to the medical service of Dr. Leo Kessel on Feb. 3, 1932. He was discharged from the hospital on Feb. 11, 1932, and returned to the admitting office on the following day, when he died of pulmonary edema.

*History.*—The patient had varicella in childhood and admitted having had two attacks of gonorrhea at the age of 17. He had married seven years before admis-

*Cases of Syphilitic Aortitis Combined with Various Types of Rheumatic Disease of the Heart*

Author	Aorta	Aortic Valve	Mitral Valve
Cabot's case of syphilitic aortitis with rheumatic myocarditis (New England J. Med. 201:177, 1929).....	Syphilitic	Normal	Normal
Dumas and Brunat (Lyon méd. 139:577, 1927) .....	Syphilitic	Normal	Rheumatic
Gallavardin and Gravier (Lyon méd. 145:541, 1930, cases 3 and 6).....	Syphilitic	Rheumatic	Normal
Gallavardin and Gravier (Lyon méd. 145:541, 1930, cases 4 and 5) and four cases in necropsy material of the Mount Sinai Hospital.....	Syphilitic	Rheumatic	Rheumatic
Stolkind <sup>1</sup> .....	Syphilitic	Syphilitic	Rheumatic
Cases 1, 2 and 3 of this report.....	Syphilitic	Syphilitic and rheumatic	Rheumatic

sion, and his wife had died about six years later from a postpartum hemorrhage. There were four healthy children. The family history was irrelevant. Eight years before, shortly after his arrival from Puerto Rico, the patient was in another hospital in New York with an attack of acute polyarthritis. Since then there had been pain and stiffness in the joints in rainy weather. There was no known cardiac involvement. He felt perfectly well until one month before hospitalization, when he noticed dyspnea and palpitation on climbing stairs. Three weeks later a non-productive cough appeared. For the past three days there had been sharp, non-radiating epigastric pain.

*Physical Examination.*—The patient appeared well developed, undernourished and chronically ill. His temperature was 99.4 F.; the pulse and respiratory rates were 96 and 20 per minute, respectively. The pupils were normal. The trachea was in the midline and freely movable. The great vessels of the neck pulsated forcibly, and a murmur could be heard over them. The cervical, bilateral axillary, inguinal and epitrochlear nodes were palpable, those in the right axilla being the size of walnuts. The lungs were normal. The heart was enlarged to the left and downward, its point of maximum impulse being located in the sixth left intercostal space 1 inch (2.54 cm.) to the left of the nipple line. A diastolic thrill was felt over the entire precordium. At the apex a blowing systolic murmur trans-

mitted to the axilla was heard; a diastolic murmur of maximum intensity was present in the second left intercostal space at the base. The second aortic sound was louder than the second pulmonic. The rhythm was regular. The radial pulses were equal, regular and markedly Corrigan in type. Pistol-shot sounds were heard over the femoral arteries, and capillary pulsation was noted. The abdomen was diffusely tender, especially in the epigastrium. The neurologic status was negative.

*Laboratory Data.*—The Wassermann and Kahn reactions of the blood were positive (4 plus). The hemoglobin was 80 per cent (17 mg. per hundred cubic centimeters of blood equals 100 per cent). There were 10,200 leukocytes per cubic millimeter of blood, of which 70 per cent were polymorphonuclear cells, 26 per cent lymphocytes and 4 per cent myelocytes. The sedimentation rate for erythrocytes was normal. The blood pressure was 154 systolic and 20 diastolic as determined on the right arm, and 130 systolic and 20 diastolic as determined on the left. The specific gravity of the urine was 1.032; an occasional trace of albumin and a few constant leukocytes were found. Roentgen examination of the chest showed a hypertrophy with marked dilatation of the left ventricle. An enlargement of the heart to the right was also noted. No abnormality was observed in the lungs.

*Course.*—The temperature rose to 101 F. soon after admission to the hospital but rapidly dropped to and remained normal as the abdominal pain disappeared. The patient was discharged as asymptomatic on the eighth day, but on the following day he became desperately ill and was brought to the hospital where he immediately died.

*Clinical Diagnosis.*—The diagnosis was chronic rheumatic cardiovalvular disease, aortic insufficiency, mitral insufficiency (and stenosis), recurrent rheumatic fever and syphilis.

*Postmortem Examination.*—This included an examination of the brain. The heart (fig. 1) weighed 625 Gm. The pericardium was smooth and glistening, except over the apex anteriorly where there was a fibrous filiform adhesion. It was enlarged markedly to the left, the apex being formed entirely by the left ventricle. The right auricle was normal. The right ventricle was dilated and hypertrophied. The tricuspid valve was normal. The pulmonary cusps were thin except for the anterior one which showed a slight localized thickening of the free edge. Toward the base of this cusp on its ventricular side were two lesions, a pink, round, slightly elevated flat-topped one about 3 mm. in diameter and another smaller, more grayish one just above. About 1 cm. above the anterior cusp in the pulmonary artery there was a flat, pink, round, finely corrugated and slightly raised lesion approximately 4 mm. in diameter. The left auricle was slightly dilated. Rising from the posterior leaflet of the mitral valve was a slightly raised, pinkish-gray corrugated roughening of the posterior wall of the left auricle. The anterior leaflet of the mitral valve was considerably thickened and vascularized. The chordae tendineae at this portion of the valve were white and thickened. The valvular insertions were broadened. There were no verrucae on the valve. The posterior leaflet was also thickened, and branching vessels could be seen on its auricular surface. The left ventricle was enormously dilated, and the chamber was globular, affecting both the inflow and the outflow tracts. The myocardium of the left ventricle was hypertrophied and grayish red. The posterior papillary muscle was markedly hypertrophied except for its apex, which was atrophied and gray. The right half of the anterior papillary muscle was almost entirely atrophied. The muscoli pectinati were rather prominent. The ventricular endocardium was gray throughout. The cusps of the aortic valve were irregularly thickened, white,

retracted and rolled inward. The edges of the leaflets and the corpora Arantii were completely inverted. There were a number of irregular thickened endocardial streaks representing early pockets of regurgitation in the subaortic region and upper outflow tract. They were also seen on the ventricular aspect of the anterior leaflet of the mitral valve. There was a slight separation of all the commissures, especially the left posterior commissure. Rising above each commissure in the aorta was a bluish-white circular lesion with scalloped edges, the surface of which was raised and irregularly corrugated. The lesions measured from 1.5 to 2 cm. in diameter. Above and adjacent to them were several small and similar lesions about 2 or 3 mm. in diameter and a larger elevation at the beginning of the transverse arch of the aorta. There was an accessory coronary ostium just anterior to that of the right coronary artery. This corresponded to the first anterior descending branch of the right coronary artery which otherwise ran a normal



Fig. 1 (case 1).—View of the left ventricular cavity. Supralvalvular syphilitic mesoaortitis with involvement and separation of the commissures and rolling and thickening of the cusps. Chronic fibrous rheumatic aortic and mitral valvulitis.

course. The left coronary orifice was normal. The orifices were not raised. There was slight atherosclerosis of the coronary arteries without narrowing of the lumen. The thoracic descending aorta showed no changes.

For histologic study in this case and case 2, blocks of cardiovalvular tissue were cut according to the standardized method described by Gross, Antopol and Sacks,<sup>5</sup> and additional blocks of tissue were cut wherever indicated. In the three cases at least two slides were stained from each block, one with hematoxylin and eosin, the other with Weigert's elastic and van Gieson's connective tissue stains.

*Microscopic Examination.*—Aorta and Left Aortic Valve: The architecture of the aorta (fig. 2A) was entirely destroyed. No clear differentiation of the

5. Gross, L.; Antopol, W., and Sacks, B.: Arch. Path. 10:840, 1930.

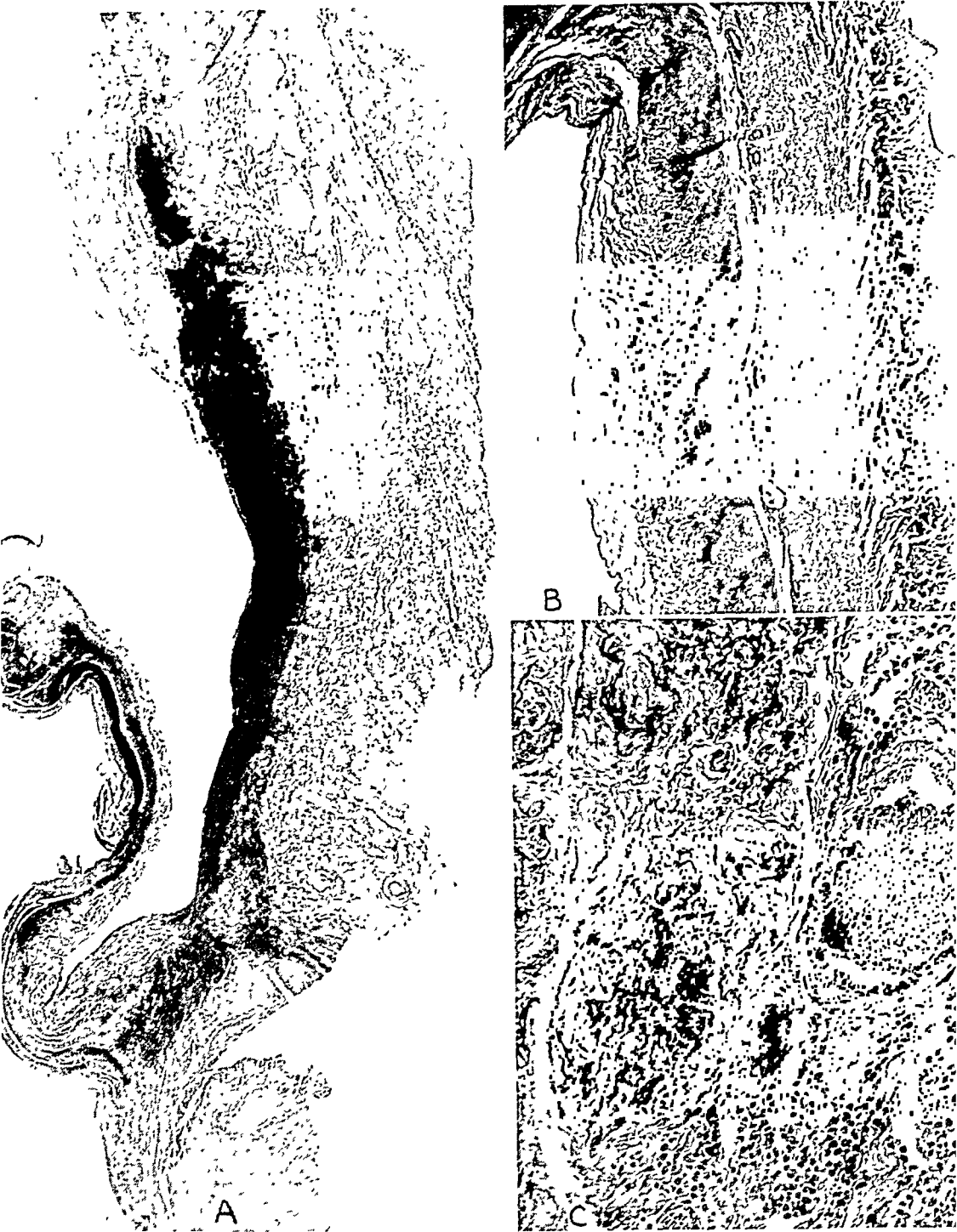


Fig. 2 (case 1).—*A*, aorta and left aortic cusp (midportion). The syphilitic mesoarteritis ends above the sinus of Valsalva and involves the adventitia of the pulmonary artery by contiguity. The valve leaflet is markedly thickened and rolled because of chronic fibrous rheumatic valvulitis. Weigert's elastic and van Gieson's connective tissue stain, reduced from  $\times 7$ . *B*, higher power of the body of the cusp in *A*, showing the vascularized broad fibro-elastic reduplications characteristic of chronic rheumatic valvulitis. Hematoxylin and eosin; reduced from  $\times 50$ . *C*, commissure between the left and right aortic leaflets showing intense syphilitic inflammation with destruction of elastica, vascularization and marked infiltration with plasma cells. Weigert's elastic and van Gieson's connective tissue stain; reduced from  $\times 200$ .

normal coats was possible. In certain areas the media was recognizable only by a few wiry twists of elastica embedded in dense richly vascularized fibrous tissue. The intima showed a typical fibro-elastic hyperplasia with little accumulation of fat, sometimes reaching a thickness in excess of that of the media. Blood vessels extended through the media into the outer zone of the intima. The adventitia was thickened and fibrous; the vasa vasorum were narrowed by an elastic hyperplastic intimal process. The vessels and a few of the nerve bundles were surrounded by dense aggregations of plasma cells, lymphocytes, endothelial cells and fibroblasts. The mesaortitis ceased abruptly at the upper margin of the sinus of Valsalva.

The adventitia of the attached pulmonary artery was similarly involved by obvious extension from the adventitia of the aorta.

The ring<sup>6</sup> of the aortic valve was densely fibrous, well vascularized and sparsely infiltrated with lymphocytes. The valve leaflet was thickened, and its free edge was knoblike. The thickening of the valve (fig. 2 B) was due to a broad musculo-fibro-elastic thickening on the ventricular side of the ventricularis layer<sup>6</sup> of elastica. The latter was also thickened and contained many bundles of smooth muscle, blood vessels with well developed muscularis and a scattering of lymphocytes. The knoblike free edge was composed of concentric whorls of dense fibrous tissue and thick elastic strands; blood vessels penetrated into the center of the knob.

The Levaditi stain revealed no spirochetes in the section.

Commissure Between the Left and Right Aortic Leaflets (fig. 2 C): The same type of lesion described in the aorta was seen. The process, however, instead of ceasing abruptly, extended unchanged in character or degree into and behind the annulus fibrosus considerably below the level of the insertion of the valve. Here there was much irregular elastic tissue showing increase and destruction, dense foci of plasma cells and arterioles with marked intimal hyperplastic thickening.

A typical perivascular Aschoff body was seen in the interventricular myocardial crest adjoining the annulus.

Mitral Valve (Posterior Leaflet): The ring<sup>7</sup> of the mitral valve was fibrosed and vascularized. There was a focal infiltration of polymorphonuclear cells and lymphocytes. The valve was markedly thickened by a hyperplastic process composed of a widened auricularis layer of elastic fibers, and on the auricular side of this layer was a wide band of collagen fibers, fibroblasts and bundles of smooth muscle. In the auricularis layer were many blood vessels with well developed muscular coats. A few perivascular Aschoff bodies were seen in the adjoining left ventricular myocardial septums.

Pulmonary Artery (Section Through the Lesion Noted in the Gross Description): In a localized area the media was distinctly thickened. The medial elastic fibers were discontinuous and showed rather abundant connective tissue between them. Blood vessels, some of which were thickened, coursed through the media

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6. In the semilunar valves the "ring" represents the proximal end of the valve leaflet constituted by the triangular ring spongiosa and adjacent portion of the fibrous annulus. The terminology of valve structures is that employed by Gross, L., and Kugel, M. A. (*Am. J. Path.* 7:445, 1931).

7. In the auriculoventricular valves the "ring" represents the proximal portion of the valve limited above by the apex of the auricular myocardial wedge and consisting of ring spongiosa and the adjacent portion of the annulus fibrosus (Gross and Kugel<sup>6</sup>).

to end in the intima, which was only slightly thickened. There were no cellular infiltrations in the middle coat. Many of the adventitial vessels revealed moderate intimal hyperplasia. There were sparse aggregations of lymphoid cells in the adventitia without particular perivascular distribution.

The peripulmonary epicardium was thickened, and there were small foci of mononuclear and plasma cells directly beneath the mesothelium.

**Pulmonary Valve** (Section Through the Nodular Lesion on the Anterior Leaflet): The ring was fibrosed and vascularized, and the annulus fibrosus was infiltrated by a few mononuclear and polymorphonuclear cells. Toward the base of the pulmonary valve, external to the ventricularis layer of elastica, there was a large hyperplastic elevation characterized by proliferation of fibrous, muscular and elastic tissue and by numerous large arterioles with extremely thick muscular walls. There was some disintegrating hemoglobin pigment beneath the endothelium. Several macrophages filled with brown iron-containing pigment were also seen here. The remainder of the valve and pulmonary artery in this section was entirely normal. There was a chronic epicarditis around the root of the pulmonary artery, as evidenced by proliferating endothelial cells, collections of plasma cells and lymphocytes, numerous young and old capillaries (many with proliferation of the lining endothelium) and a thickening of the submesothelial fibrous tissue.

**Left Ventricle:** Typical perivascular Aschoff bodies were found in the myocardial septums. There was a marked hypertrophy of the myocardial nuclei. Several arterioles showed intimal hyperplasia compromising the lumens to a slight extent. The larger coronary arteries were normal. At various points the endocardium was thickened by a vascularized musculo-fibro-elastic hyperplasia. Elsewhere there were palisade infiltrations of polymorphonuclear leukocytes, mononuclear cells and cells with gnarled and twisted nuclei.

**Left Auricle:** The left auricle was normal.

*Summary.*—The aorta showed syphilitic mesaortitis; the aortic valve, old rheumatic valvulitis and syphilitic involvement of the commissures; the mitral valve, typical old rheumatic valvulitis; the pulmonary artery, probably focal healed pulmonary mesarteritis; the pulmonary valve, focal, nodular old rheumatic valvulitis; the myocardium, typical Aschoff bodies in the left ventricle; the endocardium, rheumatic mural endocarditis of the left ventricle; the epicardium, chronic low grade epicarditis around the great vessels and at the base of the heart, probably syphilitic, by contiguity with the adventitia of the aorta.

**CASE 2.**—An unmarried American colored woman, a domestic, aged 36, was admitted to the medical service of Dr. A. A. Epstein on Sept. 2, 1926, and died September 7.

An attack of acute rheumatic fever occurred at the age of 9 years, and influenza at 28. The patient stated that she had not had venereal disease. No significant information was obtained in regard to the family history. The patient was known to have had cardiac disease since the age of 14 years. For the past twenty years she had had dyspnea on exertion, precordial pain, mild edema of the ankles and frequent sore throat. Three months before admission to the hospital all the cardiac symptoms had become aggravated and the patient was forced to go to bed. Drenching night sweats, chills and fever appeared. A slight cough and some orthopnea were noted recently with marked loss of weight and strength. Three days prior to hospitalization she began to have pain in the left hypochondrium, and the edema of the ankles subsided. Sanguineous sputum was expectorated.



*Physical Examination.*—The patient was undernourished, acutely ill, dyspneic and perspiring; her temperature was 103 F.; the pulse and respiratory rates were 120 and 26 per minute, respectively. The pupils were normal. There were forcible arterial pulsations in the neck. Dulness was present at the base of the right lung; there were a few fine crackles over this area; bronchial breathing and moist râles were heard in scattered areas of the left lung; auscultation revealed a coarse frictional rub in the left axilla. Over the fifth and sixth left intercostal spaces outside the nipple line a presystolic thrill and a sharp apex beat were palpated. Systolic and diastolic murmurs were heard at the apex; a loud diastolic murmur was also present at the base, being transmitted downward close to the left border of the sternum; at the aortic area there was, in addition, a rumbling systolic murmur. The rhythm was regular; the radial pulses were equal and Corrigan in type. The left side of the abdomen was exquisitely tender and held rigidly. The edge of the spleen was, nevertheless, palpable. The liver could not be felt. There was no edema or clubbing of the fingers. The neurologic status was negative except for sluggish ankle and knee jerks.

*Laboratory Data.*—The Wassermann reaction of the blood was negative. The hemoglobin was 40 per cent. There were 3,000,000 erythrocytes and 12,600 leukocytes per cubic millimeter of blood. Of the latter, 84 per cent were polymorphonuclear cells. A culture of the blood yielded abundant numbers of *Streptococcus anhaemolyticus* (viridans) in all the mediums. The blood pressure was 125 systolic and 50 diastolic. The specific gravity of the urine was 1.018; there were a trace of albumin and many erythrocytes and leukocytes. A bedside roentgen examination of the chest disclosed no abnormality in the lungs.

*Course.*—Two days after admission the patient became stuporous, and auricular fibrillation was observed. Death supervened three days later.

*Clinical Diagnosis.*—A diagnosis of chronic rheumatic cardiovalvular disease, mitral stenosis and insufficiency and aortic insufficiency, subacute bacterial endocarditis, infarction of the spleen and bronchopneumonia was made.

*Postmortem Examination.*—This included examination of the brain. The heart weighed 530 Gm. and was normal in shape and position. The musculature was normal in consistency. The pericardium was smooth and contained a normal amount of clear straw-colored fluid. The aorta was flexible and presented slight evidence of atherosclerosis. The visceral pericardium contained smooth, pearly-white pericardial thickenings, one situated at the anterior aspect of the right ventricle and almost 2 cm. in thickness. Several other smaller thickenings of the visceral pericardium were situated over the anterior aspect of the left ventricle near the apex and at the posterior aspect of the right ventricle. The endocardium of the right auricle was irregularly grayish white. The foramen ovale was closed. The tricuspid valve measured 8.5 cm. in circumference. At the junction of the anterior and mesial cusps there was an irregular thickening. The flaps were adherent to the wall of the right ventricle at this point. The scarlike thickening was fibrotic and contained a small amount of lime; the fibrosis extended from the junction of the flaps down on the ventricle for almost 0.5 cm. There was a small subendocardial hemorrhage on the superficial aspect of the scar; the lesion resembled an old healed endocarditis, the nature of which could not be grossly determined. The pulmonary valve was smooth, glistening and competent. The left auricle was somewhat dilated and its wall hypertrophied, measuring almost 1 cm. in thickness in places. The endocardium of the left auricle was irregularly and diffusely thickened. The posterior wall showed wrinkling. The mitral valve

measured 8 cm. in circumference. It was the seat of a healing bacterial endocarditis superimposed on an old chronic valvular disease. The flaps of the valve were thickened, fibrotic and scarred, presumably owing to an old healed rheumatic endocarditis; superimposed on this lesion were irregular, firm, grayish-white bacterial vegetations. At the junction of the two flaps along its line of closure was a flat, firm elevated bacterial vegetation. Extending from the free margin of the flaps at this point and continuing down on the adjacent chordae tendineae, was an irregular, fungoid, friable, grayish-white bacterial vegetation which extended down to the papillary muscle. The vegetation appeared to be a recent process. Along the left lateral aspect of the posterior cusp was an organized bacterial vegetation which extended from the free margins of the flap downward and involved the chordae tendineae. There were also many small flat vegetations on the anterior aspect of the posterior flap which measured 1 or 2 mm. in diameter. These vegetations were fairly well organized. The left ventricle was hypertrophied; its wall measured 2 cm. in thickness. The myocardium was dark brown and firm, and on section revealed areas of fibrosis. The aortic valve measured 6 cm. in circumference and was also the seat of a bacterial endocarditis. Situated between the right and posterior cusps and involving the commissure was a large, irregular, ulcerative endocarditic process 2 by 3 cm. in circumference. It involved the adjacent halves of the cusps and the commissure and extended downward, involving the mural endocardium of the posterior part of the outflow tract of the left ventricle for a distance of almost 1 cm. The vegetation had an ulcerative appearance; it was concave and contained irregular deposits of blood platelet thrombi. The process was situated just anterior to the undefended space, involving it only at its upper margin. The anterior cusp was thickened, and extending from the corpus Arantii there was an irregular, fanlike, friable vegetation measuring 1 by 0.7 cm. The cusps themselves were thickened and fibrotic. The left posterior commissure showed slight separation. The ascending aorta contained puckered scar formations with longitudinal wrinkling which had a pearly grayish-white color, grossly presenting the appearance of syphilitic aortitis. There were also raised irregularly corrugated lesions in the supravalvular region descending well into the right posterior commissure. The orifice of the right coronary artery was slightly narrowed. The main branches of the coronary arteries revealed no gross abnormalities.

*Microscopic Examination.*—Aorta: There were marked obliterative endarterial alterations in the vasa vasorum of the adventitia with relatively sparse infiltrations of mononuclear cells. Extensive destruction of the elastic tissue of the media was the predominant lesion. The media was largely replaced by the formation of dense scar tissue which was vascularized and infiltrated by mononuclear cells. Superimposed on this was a marked fibro-elastic proliferation of the intima containing a few capillaries.

**Right Aortic Cusp:** The tip of the cusp was disintegrated by a destructive endocarditis. There was a diffuse valvulitis and, in one place on the ventricular side, a marked proliferation of giant cells. The leaflet contained many channels which were probably vascular. The ring was the site of active inflammation with numerous thick-walled blood vessels (well developed media) and mononuclear cells.

**Left Aortic Cusp:** No bacterial vegetations were present on this cusp. The leaflet was markedly thickened and shortened and had a bulbous tip formed by large whorls of dense avascular and relatively acellular fibro-elastic tissue produc-

ing inversion of the cusp (fig. 3). The ventricularis elastica was intact although somewhat hyperplastic in its proximal half; the distal portion gave rise to the aforementioned whorls. The ring of the valve was polyvascularized and infiltrated with lymphocytes, histiocytes and large mononuclear cells. The attached section of the aorta revealed the characteristic inflammatory and degenerative lesions



Fig. 3 (case 2).—Aorta and left aortic cusp (midportion). The syphilitic mesoaortitis ends above the sinus of Valsalva. The valve leaflet is markedly retracted, thickened and everted. Details of the inflammatory ring lesion are not visible at this magnification. Weigert's elastic and van Gieson's connective tissue stain; reduced from  $\times 3.4$ .

described; the perivascular cellular infiltrations about the vasa vasorum were, however, more prominent here. It may be added that, whereas the lesions in the medial elastic tissue halted abruptly in the region of the superior limit of the

sinus of Valsalva, the cellular infiltrations of the adventitia continued down to the region behind the annulus fibrosus at the root of the valve.

**Aortic Valve Commissures:** Section through the left posterior commissure (grossly separated and uninvolved by bacterial endocarditis) revealed extension well into the commissural region of the same aortic lesion described in the aorta, with a subjacent well developed fibro-elastic, vascularized intimal hyperplasia, unassociated with evidence of bacterial endocarditis.

Section through the right posterior commissure (grossly involved by the bacterial endocarditis) showed identical involvement by the aortitic lesion. However, associated with this could be seen thrombo-endocarditic bacterial vegetations on the endocardial surface with a few deeper extensions of foci of acute purulent inflammation into the region of the annulus.

**Section Including Posterior Aortic and Anterior Mitral Leaflets:** The posterior aortic cusp showed a bulbous fibro-elastic enlargement of the tip similar to that described for the left aortic cusp, with identical vascular and cellular ring lesions. In the subaortic angle (Gross and Kugel<sup>6</sup>) was seen a sparsely vascularized and elastic hyperplastic layer continuing down the ventricular aspect of the anterior leaflet of the mitral valve. The root of the aorta was involved by the aforementioned lesions of syphilitic mesaortitis.

The anterior leaflet of the mitral valve was the seat of a bacterial endocarditis. The substance of the cusp and its ring was extensively vascularized and infiltrated with lymphocytes, histiocytes, large mononuclear cells and occasional polymorphonuclear and fibroblastic cells. The valvulitis involved all the layers of the leaflet, but implicated chiefly the auricular aspect external to the hyperplastic auricularis elastic layer.

**Tricuspid Valve:** This was normal.

**Pulmonary Valve:** The valve and artery were normal.

**Left Auricle:** A few areas of perivascular fibrosis were present in the auricular myocardium. The endocardium was rather poor in elastic fibers. The vascularized subendocardium occasionally dipped down between the auricular myocardic bundles to form wedge-shaped areas infiltrated with lymphocytes and large mononuclear cells.

**Posterior Papillary Muscle:** Marked interfascicular and perivascular scarring was present.

**Myocardium:** There was an acute diffuse and focal myocarditis with interstitial infiltrations of lymphocytes, large mononuclear cells and occasional polymorphonuclear leukocytes. A slight amount of perivascular fibrosis was noted. No Aschoff bodies were seen.

**Summary.**—The aorta showed syphilitic mesaortitis; the aortic valve, chronic rheumatic valvulitis, subacute bacterial endocarditis and a syphilitic commissural lesion; the mitral valve, chronic rheumatic valvulitis and subacute bacterial endocarditis; the myocardium, interstitial myocarditis and perivascular fibrosis.

**CASE 3.**—This case has been made available to us through Dr. W. J. Weiss and Dr. W. Antopol of the Bayonne Hospital and Dispensary. The patient, an unmarried Negro, aged 40, entered the hospital moribund and died shortly thereafter. Under the circumstances, only a sketchy history could be obtained. He had had an attack of polyarthritis fifteen years before his death. Three years later he had a purulent urethral discharge and a sore on the penis. At that time the inguinal glands suppurated and were drained. Two years before his death he was given a series of eighteen intragluteal injections, presumably for syphilis. The patient was asymptomatic until five months before admission to the hospital, when he became short of breath. Since that time he had become progressively worse,

with palpitation, orthopnea, swelling of the feet and abdomen and pain in the upper part of the abdomen. There were no precordial pain, cough or expectoration. On physical examination, he was dyspneic and orthopneic. Râles were heard diffusely throughout both lungs. A diffuse pulsation was present over the left lower part of the chest anteriorly. The heart was enlarged to the left: the apex was situated in the sixth left intercostal space at the anterior axillary line; the right border of the heart was percussed one fingerbreadth to the right of the sternum; a systolic and a diastolic murmur were heard over the entire pre-



Fig. 4 (case 3).—View of the left ventricular cavity. Supravavular syphilitic mesaortitis with involvement and separation of the left posterior commissure (arrow). Chronic fibrous rheumatic aortic and mitral valvulitis; fusion of the two remaining aortic commissures.

cordium. The cardiac rhythm was entirely irregular (auricular fibrillation). The liver was enlarged, and fluid was demonstrated in the abdomen. Operative scars were present in the right inguinal region. The patient's course was progressively downhill, and he died two days after admission, before a Wassermann test was made.

*Postmortem Examination.*—The heart weighed 600 Gm. and was enormously enlarged (fig. 4). There were some fibrous (rubbing) tags over the

base of the left auricle, the anterior surface of the right ventricle and the anterior surface of the right auricle. The right auricular cavity was considerably dilated; otherwise it was normal. The tricuspid valve showed fusion of the anterior septal commissure. Approximately at the middle of the anterior leaflet was a small vessel which descends 0.5 cm. from the base toward the free edge. The chordae tendineae to the anterior cusp were slightly thickened. The right ventricle showed marked dilatation of the inflow and outflow tracts. The pulmonary cusps were normal. The pulmonary artery at the base of the right-left commissure was slightly thickened. This was less marked at the right anterior commissure. There was a slight rolling of the center of the free edge of the right pulmonary cusp. The pulmonary artery was somewhat dilated and showed an irregular and wide distribution of delicate puckerings and also several small, slightly raised yellowish plaques with irregular borders, the largest of which was 0.5 cm. in diameter. The left auricle was markedly dilated. The endocardium was thickened. No auricular lesion was grossly detectable. The mitral valve showed a marked universal thickening with typical old rheumatic changes. The anterior leaflet was somewhat elongated. The posterior leaflet showed a rough, rather yellowish deposit on its anterior surface. The chordae tendineae were markedly thickened and fused. There was a definite stenosis of the orifice. On palpating the mitral ring the posterior commissure of the anterior leaflet presented a rubbery feel. On making sections through this region, the tissues occupying the auriculoventricular sulcus showed extensive scarring. The apex of the interventricular septum in this region showed a typical circular gumma 2 mm. in diameter. This gumma lay somewhat anteriorly, reaching almost to the endocardial surface under the mitral pocket. The entire posterior mitral pocket, from its right extremity to approximately 4 cm. toward the left, showed a yellowish puckered thickening of its endocardium which was covered with thrombus for a considerable extent. Additional sections through the auricle, mitral pocket and underlying posterior ventricle, starting from the posterior commissural region of the anterior leaflet and extending toward the left for 4 cm., showed a continuation of the extensive scarring process which involved the lower part of the auricular wedge, the auriculoventricular sulcus and a large portion of the anterior surface of the posterior wall of the left ventricle in this region. Another typical gumma was disclosed 1 cm. to the left of the one previously described. The left ventricle was dilated and hypertrophied. The aortic cusps were thickened. The right posterior and left-right commissures showed typical rheumatic fusion, but the left posterior commissure showed a typical syphilitic separation. The aorta, starting at the insertion of the annulus and continuing up through the entire arch showed a typical syphilitic process with a minimum of sclerotic change. The orifices of the coronary arteries were narrowed by the inflammatory process; the left was only slightly stenotic, while the right measured approximately 2 mm. in diameter. The aortitic process descended several millimeters into the wall of the sinuses of Valsalva and was contiguous with all three commissures, causing, as noted, a separation of the left posterior commissure. Otherwise the coronary arteries were normal.

*Microscopic Examination.*—Aorta: The architecture was markedly distorted by an infiltrating and sclerotic process. The adventitia was tremendously thickened and fibrosed, containing a considerable amount of new elastic tissue. Marked obliterative endarterial proliferation of the vasa vasorum and dense diffuse and focal perivascular and perineural aggregations of lymphocytes and plasma cells were seen. Large irregular dense fibrous scars, chiefly perivascular, were present in the media with thickening, twisting, rupture and distortion of the elastic fibers.

Innumerable capillaries with perivascular infiltrations of lymphocytes and plasma cells were likewise found diffusely in the media. A few focal areas of necrosis with nuclear débris were situated in the outer third of the media. There was a dense, avascular, noninflammatory fibro-elastic proliferation of the intima which was most marked where the medial lesions were most advanced.

**Commissure Between the Left and Posterior Aortic Cusps:** The aortitic lesion described continued unchanged into the region of the attachment of the two aortic cusps which grossly showed separation. In addition there was a moderate number of giant cells, occasionally resembling the Langhans type, in the annulus fibrosus.

**Left Aortic Cusp:** The attached segment of aorta showed the same aortic lesion descending into the wall of the sinus of Valsalva, but ending abruptly at the upper portion of the aortic annulus. The ring spongiosa of the aortic cusp contained several bundles of smooth muscle and many capillaries. The capillaries ascended in liberal numbers throughout the length of the valve cusp in the broad fibro-elastic reduplication situated on the ventricular side of the hyperplastic ventricularis elastica. Toward the closure line a moderate infiltration with lymphocytes was present in the reduplicated layer. The entire cusp was more than double its normal thickness because of the superimposed reduplication (especially at the closure line where the normal thickness was quadrupled). The adjacent crest of the left ventricular myocardium showed a moderate amount of interfascicular and perivascular fibrosis but no Aschoff bodies. The mouth of the left coronary artery was moderately narrowed by thick hyperplastic intimal thickening.

**Posterior Mitral Valve:** The leaflet was tremendously thickened by extensive fibro-elastic proliferations. The ring of the valve was fibrotic, contained many vessels, often with a hyperplastic intima, and a moderate infiltration with plasma cells and lymphocytes. At this point the auricularis elastica became remarkably hyperplastic, widened and deviated toward the ventricular side of the leaflet by the broad hyperplastic fibro-elastic reduplications, themselves the site of marked vascularization and lymphocytic infiltrations continuous with those at the ring.

The attached segment of left auricular endocardium was sclerotically thickened and slightly reduplicated. The auricular myocardium was normal. The attached crest of left ventricular myocardium showed moderate interstitial fibrosis but no Aschoff bodies.

**Mitral Valve at Posterior Commissure:** Section through the region of the yellow nodule in the valve pocket showed the valve to be thicker than in the previous portion. Identical inflammatory hyperplastic alterations were present in the leaflet. A typical blood platelet thrombus undergoing organization at the base was present in the valve pocket and was thickest where it overlay the superficially situated necrotic nodule. The nodule had a fairly homogeneous, somewhat basophilic appearance, with scattered cytoplasmic and nuclear débris. This mass of necrotic tissue was surrounded by a more or less irregular zone of granulation tissue containing many capillaries and a dense infiltration of lymphocytes, plasma cells, fibroblasts and histiocytes. Delicate fibrous strands emerged from the inner aspect of this zone to invade the central necrotic area. External to the active granulating zone was a dense, jagged, fibrous capsule which sent prolongations along the adjacent subendocardium and between the myocardial fibers. These strands were likewise infiltrated with lymphocytes and, to a lesser extent, with plasma cells. They contained abundant vessels, which in the region of the auriculo-ventricular junction, showed extreme intimal hyperplasia, often with complete obliteration of the lumen. There were no giant cells.

Extensive interfascicular and perivascular fibrosis was present in the adjacent left ventricular myocardium. No Aschoff bodies were seen.

**Left Ventricle:** The endocardium was normal, except for a few small, localized, fibrous thickenings. There was a definite excess of perivascular fibrous tissue, as well as slight, more diffuse interstitial fibrosis. No definite Aschoff bodies were found. There was moderate hyperplasia of the intima of the smaller and larger arteries, often localized or nodular. The epicardium was not thickened, but contained numerous foci of plasma cells and lymphocytes.

**Pulmonary Artery:** A section through the plaque described grossly showed definite pathologic changes in the adventitia and media. The adventitia was densely scarred by fibro-elastic connective tissue containing blood vessels with a moderate degree of endarterial hyperplasia. About these vessels were collections of plasma cells and lymphocytes. Neither the blood vessels nor the infiltrations descended into the media at this point. The media showed edematous changes between the elastic fibers. The intima was slightly thickened.

**Pulmonary Artery and Valve:** The pulmonary artery was normal. The ring spongiosa contained a few blood vessels and no cells. The tip of the valve was thickened by an increase of spongy connective tissue on the ventricular side of the ventricularis elastica, but the valve as a whole was free from inflammatory changes. The adjacent right ventricular myocardium was normal.

**Commissure Between Right and Left Pulmonary Valves:** The pulmonary artery above the commissure was normal. The wedge of media at the root of the pulmonary artery which descends into the commissure was invaded by numerous blood vessels. Those in the outer layers of the media were surrounded by plasma cells. The adventitia and media showed scarring with rupture and increase of the elastic fibers. The valvular endocardium and intima in the commissural region were thickened by dense vascularized scar tissue.

**Anterior Tricuspid Valve:** The tricuspid valve was normal, except at its insertion, where the ring was invaded by a sheaf of arterioles which descended a short distance into the body of the valve. The annulus fibrosus was slightly thickened. The myocardium was normal. The right auricle was negative. No Aschoff bodies were present.

**Summary.**—The aorta showed syphilitic (gummatous) aortitis; the aortic valve, old rheumatic valvulitis, syphilitic involvement of the left posterior commissure and aortic stenosis and insufficiency; the mitral valve, old rheumatic valvulitis and mitral stenosis and insufficiency; the left ventricle, gumma of the myocardium and interstitial fibrosis; the pulmonary artery, focal arteritis and inflammation of the commissures of the pulmonary valve; the tricuspid valve, old valvulitis; the epicardium, chronic epicarditis.

#### COMMENT

The frequency of involvement of the aortic valve in syphilitic meso-aortitis has been variously estimated at 10, 28 and 36.5 per cent (Eich,<sup>8</sup> Cabot,<sup>2</sup> and Clawson and Bell<sup>10</sup>). Such valvular involvement<sup>11</sup> generally signifies gross and microscopic descent of the inflammatory process

8. Eich, P.: *Frankfurt. Ztschr. f. Path.* **7**:373, 1911.

9. Footnote 9 deleted by author.

10. Clawson, B. J., and Bell, E. T.: *Arch. Path.* **4**:922, 1927.

11. A more detailed description is given by Saphir and Scott (*Am. J. Path.* **3**:527, 1927; footnote 12), Krischner (*Virchows Arch. f. path. Anat.* **282**:30, 1931) and Benedict (*ibid.* **281**:780, 1931).



into the aortic commissures with separation and absorption of the commissural extremities of the cusps. A less common mechanism in the involvement of the aortic valve is weakening and dilatation of the root of the aorta. In either case the free borders of the individual leaflets become too short and incapable of competent diastolic closure. As a result of the tension and friction constantly present in such incompetent valve leaflets it is common to find marked sclerosis, thickening and shortening of the entire leaflet with rolling of the free borders. This is principally true of the valvular defects associated with commissural syphilis. Although the lesion in the body of the aortic cusp may be at times grossly indistinguishable from chronic fibrous rheumatic valvulitis, the etiology of the fibrous deformed aortic valve is nearly always clear in uncomplicated cases of commissural syphilis. However, when associated with mitral stenosis, adhesive pericarditis, old left auricular endocardial lesions or any other clinicopathologic indication of rheumatic cardiovalvular disease, macroscopic estimation of the rôle played by the rheumatic virus in the production of the aortic valvular defect is impossible. Recourse must be had to microscopic investigation.

The histopathology of the aortic valve in syphilitic aortic insufficiency has been described by Saphir and Scott<sup>12</sup> who, in a series of *one hundred and seven cases of syphilitic aortic insufficiency with commissural involvement (separation)*, found inflammatory changes restricted to the commissural extremities of the cusps. The free border of the central portion of the leaflet, although frequently markedly thickened and rolled, was fibrotic, avascular and contained few if any infiltrating cells. Krischner was able to confirm this. Benedict, in a similar group of seventeen cases, presented in detail the microscopy of syphilitic aortic valves, classifying them into "ascending" (in the Monckeberg sense) and "descending" syphilitic types. The latter obviously represents extension to the valve cusp through the commissure, and here Benedict's observations are in agreement with the accepted teachings. With regard to the controversial "ascending" type of extension of the syphilitic process from the sinus wall onto the aortic layer of the leaflet (Benda<sup>13</sup> and Ribbert<sup>14</sup>), he substantiates the observations of thickening and sclerosis in the free border of the cusp, but notes, in addition, extensive formation of new capillaries, congestion and perivascular lymphocytes in the triangular expanse of the spongy connective tissue layer at the base of the

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12. Saphir, O., and Scott, R. W.: *Am. Heart J.* 6:56, 1930.

13. Benda: *Die Gefäße*, in Aschoff, L.: *Pathologische Anatomie; ein Lehrbuch für Studierende und Aerzte*, Jena, Gustav Fischer, 1911; quoted by Benedict.<sup>11</sup>

14. Ribbert: *Die Erkrankung des Endocards*, in Hencke, F., and Lubarsch, O.: *Handbuch der speciellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1930, quoted by Benedict.<sup>11</sup>

leaflet.<sup>15</sup> He regards the entire anatomic picture as indicative of syphilis. We shall refer to this later.

In order to establish definite histologic criteria for the recognition of syphilitic valvular lesions, we have investigated ten cases of syphilitic aortic insufficiency with commissural involvement in which the history and anatomic findings definitely excluded rheumatism. Microscopic examination of these thickened, deformed aortic valves (studied both near the commissure and in the midportion of the cusp) confirmed many of the observations of Saphir and Scott. At the maximum distance from the commissure inflammatory changes were entirely absent in the cusps in six cases. In three a moderate number of capillaries and round cells were found only in the ring and not in the cusp. In a single instance a more marked ring lesion consisting of thick-walled vessels with endothelial proliferation was found. This was observed to descend by contiguity from the intense syphilitic aortitis immediately above it and to ascend for a short distance into the ventricular side of the base of the leaflet. In short, only one of the ten cases showed inflammatory changes in the cusp per se. (An additional eleventh case showing well developed vascularization of the ring and the ventricular side of the entire length of the aortic leaflet was omitted because rheumatic disease could not be definitely excluded. Capillaries were present in the ring, base and closure line of the anterior cusp of the mitral valve and in the ring of the pulmonary valve. In this case the aortic valvulitis could not be traced as a direct extension from the syphilitic process in the aorta.) Marked avascular and relatively acellular fan-shaped whorls of fibro-elastic proliferation were generally present at the free border of the valve with regular noninflammatory thickening of the fibrous and elastic layers of the root of the cusp. The thickening of the root of the body of the cusp was always less than that at the free border. On the other hand, sections taken near the commissures revealed typical syphilitic chronic inflammatory alterations, most intense at the ring and tapering off as the free border was approached. Immediately adjacent to the commissures the small segment showed diffuse and most intense lesions.

In four additional cases of syphilitic aortic insufficiency showing marked fibrocalcific deforming disease of the aortic valve<sup>16</sup> capillaries and round cells were present in the cusps and rings in three instances. They bore no relation to the distance from the commissure and were apparently reactive changes to lipoid and calcareous degeneration. They are not to be interpreted as of specific inflammatory origin.

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15. This has been aptly termed ring spongiosa (Gross and Kugel<sup>o</sup>).

16. These cases will be described in more detail with a group of cases of fibrocalcific disease of the aortic valve to be published.

Although Benedict did not indicate how far from the commissure his sections in the "ascending" type were taken, it is extremely suggestive from our observations that the severe inflammatory lesions he noted in the valve rings were in close proximity to the commissures, which is not representative of the true anatomic condition of the larger midportion of the cusp. In the latter location we were able to find marked inflammatory involvement of the valve in only one of our ten cases. We therefore believe that one must expect, as a rule, to find little or no inflammatory change in this midregion. When any exists, it represents either the rare "ascending" type of extension from the root of the aorta or a horizontal diffusion from the commissures.

In contradistinction to the histologic syphilitic lesions just described are those found in this laboratory by Gross<sup>17</sup> and his co-workers in a large series of cases of chronic rheumatic cardiovalvular disease. Marked vascularization (often arterioles with well developed muscular coats) was found with remarkable constancy in the valve rings, regardless of the distance from the commissures, and in all but inactive cases lymphocytic infiltrations were also present. The aortic cusps themselves were markedly thickened by extensive irregular reduplications of fibrous tissue with varying amounts of vascularization and proliferation of elastic fibers. These layers, the site of inflammatory changes identical with those in the ring, were situated on the ventricular side of the cusp causing deviation (toward the arterial side) of the hyperplastic ventricularis layer of elastica, itself similarly inflamed.

Because of the limitation of syphilitic inflammation to the commissural extremity of the cusp<sup>18</sup> in a great majority of cases, the histologic demonstration of the previously described inflammatory lesions in the midportion of the cusp is indicative of rheumatic valvulitis, even though commissural syphilis is present. In a valve presenting both types of lesions a dual etiology must be considered—commissural syphilis and valvular rheumatism.

Reference to the protocols and figures of our three cases leaves no doubt that they fall into this group of combined syphilitic and rheumatic disease of the aortic valve, the former condition apparent at the commissure, and the latter involving the entire leaflet.

Since isolated syphilitic or rheumatic disease of the aortic valve may present identical clinical pictures, it is impossible to predict definitely the clinical concomitance of the two. When there is anamnestic and clinical evidence of both syphilitic and rheumatic infection, the existence

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17. Gross, L.: Personal communication to the authors.

18. This does not take into consideration the extremely rare cases of granulomatous or gummatous involvement of the valves of the heart, examples of which are to be published shortly.

of the physical signs of mitral stenosis or insufficiency leads one to avoid, sometimes unjustifiably, the diagnosis of syphilis of the commissures of the aortic valve. Likewise, if signs of aortic stenosis as well as of insufficiency are present in a syphilitic patient, the diagnosis would almost certainly be uncomplicated rheumatic disease of the aortic valve. (Rarely, fibrocalcific disease of syphilitic aortic leaflets may produce the clinical signs of stenosis: one might incline toward this diagnosis in the presence of a definitely syphilitic lesion at the root of the aorta, i. e., aneurysm, with clinical signs of aortic stenosis and insufficiency and without evidence of organic mitral valve or pericardial disease.)

In case 1 the clinical and laboratory data pointed to the presence of combined syphilitic and rheumatic infection. Because of the relative youth (27 years) of the patient and the predominance of the rheumatic history, the diagnosis of syphilitic valvular disease was not seriously considered clinically. For the same reasons the aortic lesion was submitted to a careful pathologic examination to exclude the possibility of its being rheumatic aortitis. However, the probability that the patient acquired syphilis ten years before death (at the age of 17, when he already had had gonorrhea), the + plus Wassermann reaction, the typical supravulvar location of the aortitis and the histologic details leave little room for doubt. A comparison of this case with one of known rheumatic aortitis, which was grossly similar, revealed no striking histologic resemblance, nor did the lesion present any of the characteristics described by Pappenheimer and Von Glahn<sup>19</sup> in rheumatic aortitis.

Case 2 offered no ground for the clinical diagnosis of syphilis. The history and results of physical examination were characteristic of rheumatic disease of the heart and the Wassermann reaction of the blood was negative. The pathologic demonstration of supravulvar syphilitic aortitis descending grossly and histologically into two of the aortic commissures was an unexpected observation.

As in case 1, the clinical findings in case 3 indicated the presence of both rheumatic and syphilitic infection. The extent to which either participated in the evolution of the cardiac syndrome was not clear clinically.

Three unusual pathologic complications in these cases warrant further mention, i. e., the lesion of the main pulmonary artery in the first and third cases, the gumma in the third case and the superimposed subacute bacterial endocarditis in the second.

The etiology of the lesions found in the pulmonary arteries in cases 1 and 3 remains unclear. In these patients the adventitial lesion, with its perivascular collections of round cells and marked intimal hyperplasia

19. Pappenheimer, A. M., and Von Glahn, W. C.: *J. M. Research* 44:489, 1924.

of the vasa vasorum, is best explained as an extension by contiguity from the severely inflamed adventitia of the aorta. However, the etiology of the scarred, acellular medial lesions seen in each of these two patients must remain undetermined, since such scars could conceivably be due to either a healed syphilitic or a healed rheumatic lesion. The same attitude must be assumed toward the commissural lesions found in the pulmonary valve in case 3, although in this patient again the location and the type of cellular infiltrations favor the diagnosis of syphilis.

Gumma of the myocardium is a rare lesion, and its presence in case 3 was an entirely fortuitous finding, unassociated with clinical manifestations. This case is also being reported elsewhere.<sup>20</sup>

It is not necessary to comment on the occurrence of subacute bacterial endocarditis in case 2, except to note that it is not a rare complication of either rheumatic cardiovalvular disease or syphilitic aortic insufficiency.

#### SUMMARY

Three cases of combined syphilitic and rheumatic disease of the aortic valve are reported. In the presence of commissural syphilis, sclerotic deformities of the aortic valve usually occur. When rheumatic lesions are found elsewhere in the heart, it is at times impossible to decide from macroscopic examination alone whether rheumatic disease participated in the production of the aortic valvular defect. By the aid of established histologic criteria for the recognition of rheumatic and syphilitic disease of the aortic valve it is possible to demonstrate such participation, if it exists. Emphasis is placed on the *limitation* of syphilitic changes in the aortic leaflets to their commissural extremities in the great majority of cases, and on the *diffuse* nature of the characteristic lesions of rheumatic valvulitis. Interstitial valvulitis in the midportion of an aortic cusp, particularly with fibro-elastic vascularized reduplications on the ventricular aspect, usually signifies disease other than syphilis (nearly always a rheumatic infection<sup>21</sup>). Association of these two types of histopathologic lesions establishes the existence of combined syphilitic and rheumatic disease in the aortic valve. The rarity of this association is indicated by the fact that it has, to our knowledge, never been previously reported.

20. In a report on rare syphilitic lesions of the heart to be published shortly.

21. In the absence of definite rheumatic manifestations elsewhere in the heart, the etiology of this type of microscopic lesion is still probably rheumatic, although in less characteristic instances it may represent healed lesions of other endocarditides.

# HODGKIN'S DISEASE

## SEARCH FOR AN INFECTIVE AGENT AND ATTEMPTS AT EXPERIMENTAL REPRODUCTION

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Since the report by Sternberg<sup>1</sup> in 1898 claiming an etiologic relationship between tuberculosis and Hodgkin's disease a voluminous and conflicting mass of evidence has accumulated. This evidence has been discussed in recent reviews by Wallhauser,<sup>2</sup> Simonds,<sup>3</sup> Vasiliu<sup>4</sup> and Chevallier and Bernard.<sup>5</sup>

Interest in the possible tuberculous origin of Hodgkin's disease was rearoused by the reports of L'Esperance,<sup>6</sup> who stated that she was able to produce tuberculosis in chickens and in previously vaccinated guinea-pigs by injection of material from persons with Hodgkin's disease. When the studies to be reported in this publication were begun there were no contradictory or confirmatory reports available. Since then Utz and Keatinge<sup>7</sup> stated that they have confirmatory evidence, which they, however, fail to give. They have already begun the treatment of Hodgkin's disease with chicken serum prepared by injection of material from tissues showing Hodgkin's disease, and they have reported encouraging results in thirty patients so treated.<sup>8</sup>

Branch<sup>9</sup> stated that certain investigators failed to confirm the experimental results of L'Esperance. Of these, Van Es investigated three cases and Aronson two cases. Schulz, on the other hand, isolated acid-fast organisms, apparently of avian type, from a node in one case. More recently van Rooyen<sup>10</sup> reported that he obtained negative results in six cases studied by cultural methods and by inoculation and grafting

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1. Sternberg, C.: *Ztschr. f. Heilk.* **19**:21, 1898.
2. Wallhauser, A.: *Arch. Path.* **16**:522 and 672, 1933.
3. Simonds, J. P.: *Arch. Path.* **1**:394, 1926.
4. Vasiliu, T.: *Ann. d'anat. path.* **8**:815, 1931.
5. Chevallier, P., and Bernard, J.: *La maladie de Hodgkin*, Paris, Masson & Cie, 1932.
6. L'Esperance, E.: (a) *J. Immunol.* **16**:37, 1929; (b) **18**:127 and 133, 1930; (c) *Ann. Surg.* **93**:162, 1931.
7. Utz, L., and Keatinge, L.: *M. J. Australia* **1**:397, 1931.
8. Utz, L., and Keatinge, L.: *M. J. Australia* **1**:521, 1932.
9. Branch, A.: *Arch. Path.* **12**:253, 1931.
10. van Rooyen, C. E.: *Brit. M. J.* **1**:50, 1933.

into fowls. Garrod<sup>11</sup> in one case was unable to produce tuberculosis in pigeons. Wallhauser<sup>2</sup> and H. L. Stewart<sup>12</sup> were unable to find evidence of tuberculosis in chickens into which the diseased tissues had been injected.

F. W. Stewart and Doan<sup>13</sup> recently presented immunologic evidence suggesting a relationship to avian tuberculosis. They found that the precipitin titer with tuberculophosphatide in known cases of Hodgkin's disease was within the range observed in known cases of tuberculosis, and that the titer with avian tuberculophosphatide tended to be higher than that with human tuberculophosphatide. These results were not obtained with other types of lymphadenopathies.

The present experiments were designed (1) to test the diseased tissues in a series of fifteen cases of Hodgkin's disease (as well as in eight control cases of different lymphomas) for ability to produce tuberculosis in chickens, guinea-pigs, rabbits, dogs and mice, and (2) to test these same tissues by special cultural methods for the presence of tubercle bacilli. In each case of Hodgkin's disease the diagnosis had been established by examination of microscopic sections of the diseased tissues by two or more experienced, disinterested pathologists, who used as their criterion the classic description by Reed.<sup>14</sup> Lack of space precludes a description of each case in detail. Most of the patients were followed clinically. In the evolution of their disease and in their symptoms, physical signs, response to roentgen therapy and other clinical manifestations they presented typical Hodgkin's disease. Because of the scope of the investigation, the significant but negative results obtained and the objections raised by certain critics to the procedures used by other investigators, the experimental procedures will be given in detail.

#### EVIDENCE FROM INOCULATIONS AND TRANSPLANTATIONS OF THE DISEASED TISSUES INTO ANIMALS

Experiments were planned to determine the transmissibility of possible infectious agents, whether bacteria or virus, and the transplantability of tissues which may possibly be regarded as neoplastic.

Material from 15 persons with proved Hodgkin's disease was inoculated into 35 chickens, 24 guinea-pigs, 18 rabbits, 7 dogs and 3 mice. Tissues secured at autopsy from suspicious lesions in these animals were in turn used in further attempts at transmission or passage of the disease in 4 chickens, 4 guinea-pigs and 4 rabbits.

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11. Garrod, L. P., in *Rose Research on Lymphadenoma*, Bristol, John Wright & Sons, Ltd., 1932, p. 105.

12. Stewart, H. L.: *J. Lab. & Clin. Med.* **18**:281, 1932.

13. Stewart, F. W., and Doan, C. A.: *Ann. Surg.* **93**:141, 1931.

14. Reed, D. M.: *Johns Hopkins Hosp. Rep.* **10**:133, 1902.

Similarly conducted control experiments were made in 8 cases of different types of lymphadenopathy. These included 3 cases of lymphosarcoma (1 of reticulum cell type and 2 of lymphocytic cell type), 3 cases of leukemia (1 each of aleukemic myelosis, aleukemic lymphadenosis and chronic lymphatic leukemia) and 2 cases of tuberculous lymphadenitis. These cases were chosen because they were indistinguishable from cases of Hodgkin's disease by their clinical features or by their blood pictures. The biopsy or the subsequent course determined the diagnosis. Inoculations were made into 22 chickens, 15 guinea-pigs, 14 rabbits, 1 dog and 9 mice. Tissues from some of these animals in turn were used in attempted passage of the disease in 7 chickens, 6 guinea-pigs and 6 rabbits. Thus material in 23 cases was injected into 199 animals of different species.

A second type of control consisted of 23 chickens that were not subjected to injections. This group was chosen to provide a check on the occurrence of spontaneous tuberculosis—reputedly common in this species—and on environmental factors. Representative specimens were selected from successive lots of chickens purchased. They were kept in cages alternating with those of the fowl given injections and were subjected to the same environmental conditions.

*Experimental Procedure.*—The lymphogranulomatous and control tissues were secured aseptically by biopsy or at autopsy. A representative portion of each lymph node or spleen used for injection was prepared for histologic examination. The remainder was chopped and ground in a sterile mortar with sterile physiologic solution of sodium chloride. When the tissue was fibrous the maceration was aided by the use of sterile sand. The suspension was injected as soon as possible, in some instances within two hours after the removal of the tissue. Intravenous injections were made through a 23 gage hypodermic needle. Subcutaneous, intraperitoneal and intracerebral injections were made through a needle of 20 gage or larger. An attempt was made to inject particles that would barely pass through the needles. On the whole rather large doses were used (tables 1 and 2).

Partly grown chickens of both sexes were used. At first they were purchased in the open market. After a tuberculous chicken had been found, all were subsequently purchased directly from a farm. The chickens had been raised in brooders on an open range and were shipped to the laboratory in crates previously unused. In addition, they were tuberculin-tested and kept away from known sources of accidental infection. The conditions of food and environment were not ideal. Certain chickens showed signs of mineral and vitamin deficiency and should have been readily susceptible to infection. After receiving injections they were kept in cages 3 tiers high. Thus there was opportunity for infection of those in the bottom rows with material dropped from those above. The quarters were rather dark, no direct sunlight being admitted. There was little opportunity for exercise. Every chicken received an injection in the wing vein from the suspension described. It was found that despite an occasional immediate death from embolism chickens tolerate coarse suspensions better than do rabbits. In addition to the intravenous injections some chickens were given subcutaneous and intraperitoneal injections.



TABLE 1.—*Experiments in Which Human Tissues Affected with Hodgkin's Disease Were Injected into Animals*

Case	Sex of Patient	Age	Source of Material	Kind of Material	Amount, Gm.	Injections				Animals That Became Tuberculous	Reinjections				Animals That Became Tuberculous
						Guinea-Pigs	Rab-bits	Chick-ens	Dogs	Mice	Diseased Chickens	Guinea-Pigs	Rab-bits	Chick-ens	
1	M	13	Biopsy	Axillary	2.4	2	1	2	..	..	....	..	..	..	None
2	M	52	Autopsy	Retropertoneal	10.0	2	2	2	..	..	O 4	2	2	2	None
4	M	8	Autopsy	Retropertoneal	10.0	3	3	3	..	..	ChS	2	2	2	Chicken 18
5	M	27	Biopsy	Cervical, axillary	2.0	3	2	3	..	..	....	..	..	..	None
7	M	30	Biopsy	Cervical	1.0	..	..	3	..	..	....	..	..	..	None
8	F	24	Biopsy	Cervical	0.5	..	..	2	..	..	....	..	..	..	None
10	M	14	Biopsy	Cervical	....	2	2	3	..	..	....	..	..	..	None
12	M	10	Autopsy	Splenic, tracheal, retro-peritoneal	10.0	2	2	3	1	..	....	..	..	..	None
13	M	28	Biopsy	Cervical	2.0	2	2	3	1	..	....	..	..	..	None
14	M	30	Biopsy	Cervical	0.5	..	..	3	1	3	....	..	..	..	None
15	F	19	Biopsy	Axillary	1.0	..	2	2	1	..	....	..	..	..	None
17	M	21	Biopsy	Cervical	0.5	2	..	2	..	..	....	..	..	..	Guinea-pig 47
21	F	31	Biopsy	Axillary	3.5	2	2	2	..	..	....	..	..	..	None
22	M	37	Biopsy	Cervical	0.6	2	..	..	2	..	....	..	..	..	None
23	F	24	Biopsy	Cervical	1.0	2	..	2	1	..	....	..	..	..	None
Total (15 cases).....						24	18	35	7	3		4	4	4	

TABLE 2.—Control Experiments

TABLE 2.—Control Experiments

Sex of Case	Patient	Age	Source of Material	Kind of Material	Amount, Gm.	Injections					Animals That Became Tuberculous	Reinjections				Animals That Became Tuberculous
						Amount, Gm.	Guinea-pigs	Rabbits	Chickens	Dogs		Mice	Diseased Chickens	Guinea-pigs	Rabbits	
Tuberculous adenitis																
11	M	20	Biopsy	Cervical	4.0	2	2	3	..	2	2 guinea-pigs, 2 mice	..	2	2	2	None
16	F	63	Biopsy	Cervical	0.5	..	..	2	..	..	None	....	..	..	..	None
Lymphosarcoma																
3	M	50	Autopsy	Retropertoneal	10.0	3	2	3	..	..	1 guinea-pig	C13 C15	2 2	2 2	2 2	None None
9	M	40	Autopsy	Splenic, retro-peritoneal	....	2	2	3	1	3	None	....	..	..	..	None
19	F	79	Autopsy	Retropertoneal	11.0	2	2	3	..	1	None	....	..	..	..	None
Leukemia																
6	M	27	Autopsy	Splenic, peritoneal	....	2	2	3	..	..	None	....	..	..	..	None
18	F	51	Biopsy	Cervical	1.0	2	2	2	..	..	None	....	..	..	..	None
20	M	72	Autopsy	Retropertoneal	....	2	2	3	..	..	None	....	..	..	..	None
Controls (no injections given).....						..	..	23	..	..	None	....	..	..	..	None
Total (8 cases).....						15	11	15	1	9			6	6	6	

The rabbits and guinea-pigs received the usual laboratory care. They were kept in hygienic surroundings away from tuberculous animals. Young animals were used and they gained weight rapidly. They were also tuberculin-tested before use. The rabbits were given intraperitoneal injections. In addition, some were given subcutaneous injections. The guinea-pigs usually received subcutaneous injections, but some also received intraperitoneal injections. Transplantation of thin wafers of tissue was made subcutaneously and intraperitoneally into some guinea-pigs and rabbits.

Each dog received an intracerebral injection through a small perforation of the skull in the frontal region. Since tuberculous dogs do not give a cutaneous reaction<sup>15</sup> to tuberculin this test was often omitted. The intracerebral injections were made because of the success reported by Feldman<sup>16</sup> in infecting dogs with avian tubercle bacilli by this method.

After the injections had been given, all animals were watched carefully for signs of disease. They were weighed frequently. Tuberculin tests were performed frequently on the chickens, both avian and human tuberculins of proved potency being used. Old tuberculin 50 per cent of full strength was used in 0.1 cc. doses. At the termination of the experiment some chickens had received seven different tests with each tuberculin. There was no evidence that any of them had been sensitized by the tuberculin of previous tests. The rabbits and guinea-pigs were tuberculin-tested less frequently, but all were tested before the end of the experiment. For these tests, decreasing dilutions of human and avian tuberculins down to that of 1:10 were used.

The animals that did not spontaneously succumb to disease were killed by overanesthetization between the ninth and thirteenth months. The dogs were an exception in that these experiments were terminated between one hundred and two hundred and seventy-four days. All animals were carefully examined grossly at autopsy for evidence of disease. Tissues were then preserved in fixing solution and sections were examined as a routine from lungs, liver, spleen and kidneys as well as from other organs as indicated. All tissues that were tuberculous or that contained lesions even remotely suggesting a granuloma were stained for acid-fast organisms. For this purpose, the Cooper<sup>17</sup> modification of the Ziehl-Neelsen stain was most satisfactory.

Certain animals at autopsy presented lesions the nature of which was uncertain. The tissues showing these lesions were subjected to further study by three methods: 1. Direct smears were stained by the Ziehl-Neelsen method. 2. Cultures were made according to the method described for growing the tubercle bacillus. 3. Passage experiments were carried out (tables 1 and 2) in which these tissues were used for injection into other animals, the methods being used which were employed with the original material.

*Results.*—The results of the experiments with tissues from persons with Hodgkin's disease are given in table 1; those of the experiments with the control materials, in table 2, and a summary, in table 3. Several experiments deserve special comment.

Of the 35 chickens that received injections of suspensions of tissues from persons with Hodgkin's disease and 22 that received injections

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15. Steiner, P. E.: *Am. Rev. Tuberc.*, to be published.

16. Feldman, W. H.: *Am. J. Path.* 7:147, 1931.

17. Cooper, F. B.: *Arch. Path.* 2:382, 1926.

of suspensions of control tissues, only 1 died of undisputable tuberculosis. In this experiment 3 guinea-pigs, 3 rabbits and 3 chickens received injections of the same retroperitoneal lymph node suspension. Of the 9 inoculated animals, only 1 chicken acquired tuberculous lesions. The patient, a boy 8 years of age, died two years after the onset of symptoms referable to Hodgkin's disease (case 4). One month after injection of the material the tuberculin reaction in this chicken (C 18) was strongly positive. The fowl lost over half of its original weight and died, nine weeks after inoculation, with a massive tuberculous infection of the liver, spleen and lungs. On histologic examination the acid-fast bacilli were present in great numbers. Unfortunately, spontaneous

TABLE 3.—*Summary: Transplantations and Injections into Animals; 222 Experiments*

Diagnosis of Condition of Patients from Whom Tissues Were Taken for Injection into Animals	No. of Cases	Guinea-Pigs		Rabbits		Chickens		Dogs		Mice	
		Injections	Tuberculous	Injections	Tuberculous	Injections	Tuberculous	Injections	Tuberculous	Injections	Tuberculous
Hodgkin's disease.....	15	24	1	18	0	35	1†	7	0	3	0
Reinjections (passage).....	(2)	4	..	4	..	4					
Injected controls:											
Lymphosarcoma.....	3	7	1	6	0	9	0	1	0	7	0
Reinjections (passage).....	(3)	4	..	4	..	5					
Leukemia.....	3	6	0	6	0	8	0				
Tuberculous adenitis.....	2	2	2	2	0	5	0	..	..	2	2
Reinjections (passage).....	(1)	2	..	2	..	2					
Controls not given injections.....	..	..	..	..	..	23*					
Total.....	23	49	4	42	0	91	1	8	0	12	2

\* These controls were not given injections but were kept in cages alternating with those of the chickens given injections and were subjected to the same environmental conditions.

† One of the chickens given injections.

tuberculosis cannot be ruled out. This chicken was one of the first in the series and was not tuberculin-tested prior to the injection of the suspension of tissue from the patient with Hodgkin's disease. A suspension of the liver and spleen of this chicken was injected into 2 chickens, 2 rabbits and 2 guinea-pigs. One chicken died with tuberculosis, principally of the liver, in six weeks, and the other, of tuberculosis of the liver, spleen, lungs and bone marrow in nine weeks. One of the guinea-pigs died in six weeks with marked generalized tuberculosis. The second guinea-pig acquired a positive reaction to avian tuberculin but failed to react to human tuberculin; when killed ten months after injection it had only a few scars in the lungs, which on histologic examination could not be definitely identified as tuberculous. Of the 2 rabbits, one died two weeks after receiving the injection, during a minor epidemic of enteritis, and revealed no sign of tuberculosis, while the other died

in six weeks of extensive tuberculosis. Cultures of the original chicken liver and spleen on various mediums yielded acid-fast bacilli which on injection into chickens killed them with typical tuberculosis.

Many other chickens died during the course of the experiments. Perhaps the most common cause of death was leukemia or a leukemoid reaction. Such chickens did not acquire a positive reaction to tuberculin, although occasionally a slight edematous swelling occurred at the test site. They did not lose much weight, and at autopsy the gross observations were not those of typical tuberculosis. Histologic examination revealed the nature of the disease. There was an absence of stainable acid-fast bacteria in such lesions, cultures for acid-fast organisms were negative and in several passage experiments no tuberculosis was produced. Furthermore such leukemoid reactions occurred in the control groups of chickens, both those given and those not given injections.

Another lesion worthy of special mention which was encountered 4 times was a granuloma of the wall of the gastro-intestinal tract, usually in the cecum. These masses grossly resembled one described by L'Esperance<sup>6a</sup> as tuberculous. Microscopically, however, they showed varying amounts of new granulation tissue and fibrous connective tissue. There were areas of massive necrosis and also smaller necrotic foci, at the periphery of which there was sometimes a marked giant cell reaction. The chickens producing the granulomas lost much weight. They had persistently negative reactions to tuberculin. No acid-fast bacteria were ever seen in multiple stained sections and no growth of acid-fast organisms was recovered in cultures. Passage experiments failed to produce tuberculosis in the various species of animals used. Two chickens in which such tumors developed were *controls not given injections*; another was a control given an injection of a suspension of lymphosarcomatous tissue, and the fourth was a chicken used in a passage experiment in which a suspension of tissue from a leukemic chicken was injected. These lesions are therefore considered to be nontuberculous.

Lesions of still another type were encountered in 3 chickens. These consisted of firm, grayish-yellow, discrete but unencapsulated nodules in the liver, measuring from 4 to 10 mm. in diameter. On histologic examination the nodules were seen to be made up of many types of cells, among which small and large mononuclear cells predominated. There were numerous cells resembling myelocytes, as well as some large mononuclear giant cells. Cells with a granular, eosinophilic cytoplasm were numerous. Cells of the type of the plasma cell were also present. All these cells were supported in a reticular framework which resembled that of the original liver (fig. 1). In addition to these features common to all, one nodule showed several focal accumulations of densely packed cells with small round and oval nuclei with an even chromatin pattern and a very eosinophilic, finely granular, moderately abundant cytoplasm.

Immediately surrounding these foci were found rings of endothelioid giant cells with clusters of pale nuclei and indefinitely outlined cytoplasm (fig. 2).

As far as one can determine from an examination of photomicrographs, these lesions show a remarkable resemblance to those illustrated by L'Esperance.<sup>6a</sup> However, I do not consider them tuberculous for the following reasons: No acid-fast organisms were demonstrable in them. The chickens on repeated tests never gave a positive reaction to tuberculin. Moreover, two of the lesions occurred in *controls that had not had injections*. The third was found in an apparently healthy

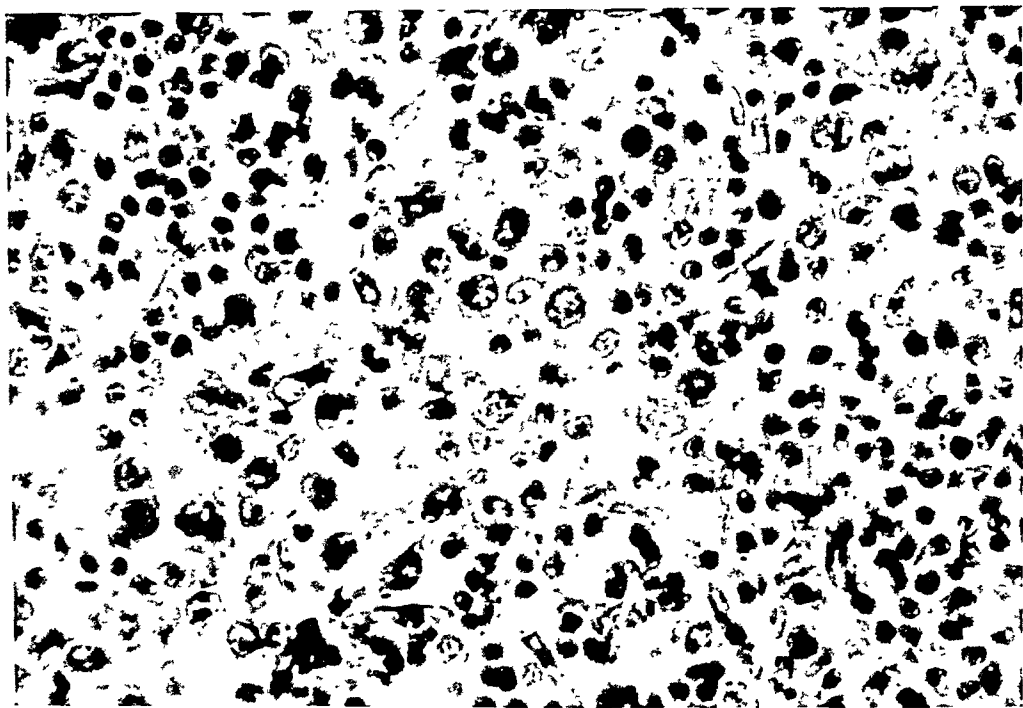


Fig. 1.—High power photomicrograph showing the types of cells found in the hepatic nodules.  $\times 1450$ .

chicken killed nine months after it had received an injection of a suspension of tissue from the patient in case 13, one of Hodgkin's disease. The other 2 chickens given injections of the same material and examined post mortem at the same time showed no disease. None of the lesions of this type showed any tendency to dissemination or to production of a fatal disease. Unfortunately, no passage experiments were performed with these lesions.

The other chickens that died before the termination of the experimental period showed a variety of lesions. They failed to show positive reactions to tuberculin or evidence of tuberculosis. Five chickens that received injections of suspensions of tissue from 2 persons with tuber-

culous adenitis presented no sign of tuberculosis. Likewise those given injections of tissues from persons with leukemia and lymphosarcoma did not become tuberculous.

Of the 24 guinea-pigs into which injections or transplantations of Hodgkin's tissues were made, only 1 contracted tuberculosis. This animal (G-P47) presented enlargement of the inguinal lymph nodes on the side of the injection and when tested four months later had a positive reaction to human tuberculin and a negative reaction to avian tuberculin. The original lymph node obtained for biopsy from the cervical region of a man 21 years of age showed no microscopic evidence

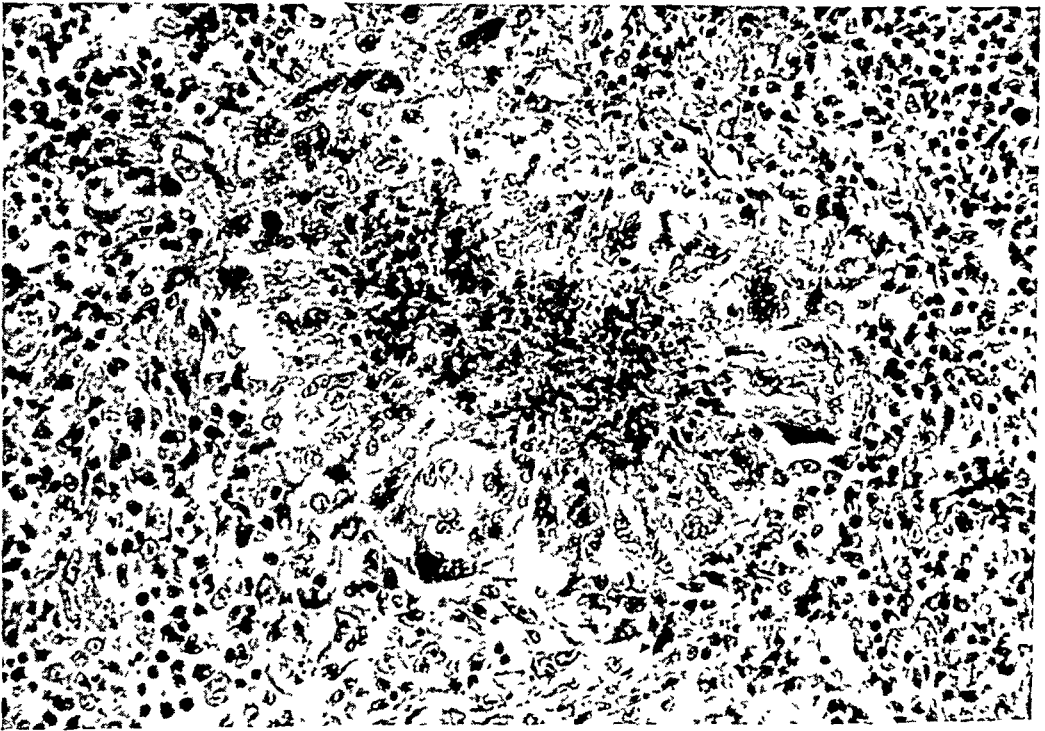


Fig. 2.—Type of lesion, resembling a tubercle, found in the hepatic nodule shown in figure 1. This is considered nontuberculous.  $\times 500$ .

of tuberculosis (case 17). The animal died six and one half months after it received the injection, with extensive tuberculosis of the lungs, liver, spleen and lymph nodes. The other guinea-pig and the 2 chickens that received injections of the same material never showed positive reactions to tuberculin and at the termination of the experiment showed no evidence of tuberculosis.

Two guinea-pigs that received injections of a suspension of tissue from one of the control patients with tuberculous adenitis died of tuberculosis. Of 13 guinea-pigs that received injections of material from 3 persons with lymphosarcoma and 3 with leukemia, 1 developed tuberculosis (case 3). This animal (G-P17) died of marked generalized

tuberculosis five months after tissue from a retroperitoneal lymph node had been engrafted into it. The node showed the histologic picture of a reticulum cell type of lymphosarcoma. No special significance was attributed to this observation since the injection of nontuberculous lymph node tissue from this location not infrequently produces tuberculosis in animals.

No rabbit, not even the 2 given injections of the material from the 2 patients with tuberculosis, contracted tuberculosis during the experiments. Seven dogs received intracerebral injections of suspensions of tissue from persons with Hodgkin's disease and one, of a suspension of lymphosarcomatous tissue, without showing evidence of tuberculosis. Of the mice that became tuberculous during the experiments, both had received injections of material from a person with tuberculous adenitis.

Because of the success of Nungester<sup>18</sup> in enhancing the virulence of bacteria by suspending them in sterile gastric mucin, this method was tried in 1 case of Hodgkin's disease. One dog and 2 guinea-pigs failed to acquire tuberculosis after being given injections of the diseased tissue suspended in mucin.

#### EVIDENCE FROM CULTURES

Cultural studies were undertaken, not primarily to determine the bacterial flora of the tissues from the patients with Hodgkin's disease, but to demonstrate acid-fast organisms if they were present. Many of the reported bacteriologic studies on lymphogranulomatous tissues antedate the development of cultural methods and procedures which today are highly recommended for the isolation of acid-fast bacteria. Thus the cultural studies served as a control of the injections and permitted application of some of the reputedly superior methods for the isolation of tubercle bacilli.

An attempt was also made to repeat the observations of Busni.<sup>19</sup> She reported that from tissues showing Hodgkin's disease she was able regularly to grow bacteria which, when examined at from twelve to twenty-four hours, were acid-fast and resembled Koch's bacillus. After another period of from twelve to twenty-four hours they appeared as small nonacid-fast cocci. After further incubation, at a time when macroscopic growth was not yet evident, they resembled masses of staphylococci. Special procedures were devised to make analogous observations on cultures concerned here.

*Experimental Procedure.*—Fragments of diseased spleen and lymph node tissues were inoculated into culture mediums as soon as possible after they were obtained for biopsy or at autopsy. This was usually within a few hours after their removal.

18. Nungester, W. J.: *Proc. Soc. Exper. Biol. & Med.* **30**:120, 1932.

19. Busni, N.: *Virchows Arch. f. path. Anat.* **268**:614, 1928.



Thin, wafer-like slices or macerated tissues were transferred to the surface of solid mediums in culture tubes. In some cases contamination was suspected and the tissues were treated with 6 per cent sulphuric acid, 5 per cent ovalic acid or 3 per cent acetic acid for thirty minutes or more before inoculation. The mediums used were Sweany's<sup>20</sup> veal, cream, milk, egg, medium, Corper's potato<sup>21</sup> and Herrold's egg yolk medium.<sup>22</sup> The latter was modified in a few instances by the addition of 5 per cent glycerin. Loeffler's serum, blood agar and Petroff's egg medium were also used at various times. Numerous tubes were inoculated with material from each case. Incubation was continued for periods up to nine months.

It is generally recognized that partial carbon dioxide tensions are superior for the isolation of some acid-fast bacteria. Accordingly some of the culture tubes were incubated in 10 per cent carbon dioxide tensions at 37 C. Since lower temperatures have been advocated as advantageous for certain strains, some tubes were kept at room temperature. The criteria for sterility have been modified by the pioneer work of Hauduroy<sup>23</sup> on serial plate transfers. With certain specimens that appeared sterile after prolonged incubation his technic was employed for periods up to twenty days before the tubes were discarded as sterile. In an attempt to repeat the observations of Busni, examinations of apparently sterile cultures were made at twenty-four hour intervals by grasping the implanted tissue in the culture tubes with sterile forceps, touching the surface which was in contact with the culture medium to the surface of a sterile glass slide, and then examining the slide preparations after staining them by the Ziehl-Neelsen and Gram methods.

*Results.*—No cultures of definitely acid-fast bacilli were obtained by these methods from tissues in 11 cases of Hodgkin's disease. Although the mediums were not especially suitable for the growth of the diphtheroids so frequently recovered by many investigators, bacteria of this type were grown in 4 cases, and other common bacteria usually considered to be contaminants were grown occasionally. In 2 cases the culture tubes remained sterile.

In only 2 instances were observations made resembling those reported by Busni. From a cervical lymph node removed for biopsy in a case of Hodgkin's lymphogranulomatosis, implanted on modified Herrold medium and incubated in 10 per cent carbon dioxide tension, small nonacid-fast bacilli of medium size appeared on the impression slide preparations. Observation of these organisms revealed that most of the body was gram-negative but that it contained from one to four, usually two, gram-positive granules of a diameter equal to that of the bacillus. The organisms containing four granules appeared to be short chains of streptococci. When only two granules were present the appearance suggested a diplococcus. After another forty-eight hours of incubation rather small, slightly beaded bacilli resembling diphtheroids, but with a tendency to be acid-fast, were numerous. Macroscopic evidence

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20. Sweany, H. C., and Evanoff, M.: *Am. Rev. Tuberc.* **18**:661, 1928.

21. Corper, H. J.: *J. A. M. A.* **91**:371, 1928.

22. Herrold, R. D.: *J. Infect. Dis.* **48**:236, 1931.

23. Hauduroy: *Compt. rend. Soc. de biol.* **97**:1392, 1927.

of growth first appeared on the ninth day, and an organism resembling a diphtheroid, without any tendency to be acid-fast, slowly grew out.

In another case the sterile slide impressions prepared after twenty-four hours of incubation of tissue secured for biopsy from a cervical lymph node in a case of Hodgkin's disease showed clusters of gram-positive coccus-like organisms. These, however, failed to become grossly visible, and although the rapid serial plate technic was used, no bacterial colonies were ever obtained.

Five controls consisting of tissues from 3 patients with lymphosarcoma and 2 with leukemia yielded no significant organisms. No acid-fast bacteria were grown from them. Diphtheroids were grown in 1 case and staphylococci were grown in 2.

#### COMMENT

In this intensive search for tubercle bacilli in tissues from patients with Hodgkin's disease by inoculation of the tissues into a variety of species of animals and by cultural methods little evidence of such organisms was found. No acid-fast strains resembling tubercle bacilli were grown by cultural methods, and inoculation of material from fifteen patients with Hodgkin's disease into animals gave tuberculosis in only 1 guinea-pig and 1 chicken. In the latter animal a spontaneous tuberculous infection was not ruled out. In neither of these cases were other animals infected by injections of the same material.

If the results of Dolgopol<sup>24</sup> showing that chickens are insusceptible to infection by the human type of tubercle bacillus are accepted—and this observation is confirmed in the 2 control cases of tuberculous adenitis used in this series—it becomes apparent that the 1 chicken that contracted tuberculosis in these experiments was infected with tubercle bacilli probably of avian type. This is substantiated by the results of injection of infected tissues and of cultures of acid-fast bacilli obtained from this chicken into other chickens.

One important factor in the propagation of tuberculosis in animals is the size of the infecting dose. This was borne in mind throughout the investigation. Consequently, in many experiments, large amounts of the diseased tissues were macerated and thick suspensions were prepared. Of these, large amounts were injected (see table 1). Nevertheless the incidence of infection remained low.

Another important factor is the resistance of the test animals. Overcrowding, poor ventilation, dark quarters, undernourishment and vitamin deficiencies lead to a higher incidence of infection. Among my experimental animals the chickens alone were subjected to some of these adverse conditions. Still they failed to present tuberculosis. This leads to the belief that acid-fast organisms pathogenic for chickens were

24. Dolgopol, V. B.: *J. Infect. Dis.* **49**:216, 1931.

extremely infrequent in, or entirely absent from, the tissues of the patients with typical Hodgkin's disease whom I studied.

Ewing<sup>25</sup> emphasized the factor of duration of time in the production of tuberculosis with such tissues. Although many of my experiments were allowed to continue for over one year there was no increased incidence of infection.

The incidence of tuberculous infection in guinea-pigs in this series was also smaller than that reported by some investigators. One guinea-pig of the 24 given injections of the tissues showing Hodgkin's disease was infected. This incidence closely approaches that reported by Twort<sup>26</sup> and by Gordon.<sup>27</sup> The latter produced tuberculosis in 3 of 40 guinea-pigs subjected to injection.

The diphtheroids so frequently grown in cultures of tissues from persons with Hodgkin's disease who have not been treated become increasingly difficult to recover from tissues irradiated by roentgen rays. It is not known whether the destruction by irradiation also applies to the tubercle bacillus. To avoid this possibility, many tissues that had never been irradiated by roentgen rays were used. This apparently had no influence on the final result.

During the examination of smears from diseased tissues stained by the Ziehl-Neelsen method a phenomenon was noticed which at first was confusing. Acid-fast granules and *débris* suggestive of degenerated bodies of tubercle bacilli were often seen. In sections of these same tissues stained by the Cooper method the nature of the acid-fast materials became apparent. A restudy of the original smears then supported the opinion that they were either granules from eosinophilic leukocytes or *débris* resulting from the destruction of red blood cells. The cytoplasm of the latter was found to be unusually tenacious for the fuchsin stain.

#### SUMMARY

Diseased splenic and lymph node tissues from 15 patients with Hodgkin's disease (lymphogranulomatosis) together with control diseased tissues from 8 patients with other lymphomas were investigated (1) for the ability of the diseased tissue to produce tuberculosis in chickens, guinea-pigs, rabbits, dogs and mice, (2) for the disease-producing capacity of these tissues as grafts, and (3) for the presence of tubercle bacilli or atypical acid-fast bacteria in these same tissues, special cultural methods being used. The diseased tissues from the 23 patients were

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25. Ewing, J.: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 407.

26. Twort, C. C.: *J. Path. & Bact.* **33**:539, 1930.

27. Gordon, M. H., in *Rose Research on Lymphadenoma*, Bristol, John Wright & Sons, Ltd., 1932.

injected or transplanted into 199 animals of the species mentioned. An additional group of 23 chickens that were not given injections served as a control of environmental factors, especially of the occurrence of spontaneous tuberculosis. A group of 8 dogs received intracerebral injections. The animals were allowed to survive for periods varying from nine to thirteen months. The criteria used for the diagnosis of tuberculosis in the animals following injection were: (1) the acquisition of a positive reaction to tuberculin, (2) the gross and microscopic morphologic structure of tuberculous lesions, (3) the presence of stainable acid-fast bacilli in suspicious lesions, (4) the growth of acid-fast bacilli from such lesions on culture mediums and (5) the production of tuberculosis in animals in passage experiments.

In the entire group of animals given injections of diseased human tissues, tuberculosis occurred in 1 chicken following injection of material from a patient with Hodgkin's disease, 1 guinea-pig following injection of tissue from a patient with Hodgkin's disease, and 1 guinea-pig following injections of lymphosarcomatous tissue. In the infected chicken a spontaneous infection was not ruled out. In passage experiments with tissues from these infected animals tuberculosis was again produced.

Numerous lesions in diseased chickens superficially suggesting tuberculosis were considered nontuberculous because they did not definitely satisfy *any* of the five criteria previously laid down.

No evidence was found that the diseased human tissues were transplantable or that they were capable of inducing in animals lesions with a similar histologic structure.

No strains of acid-fast bacteria were grown from these diseased human tissues by modern cultural methods. Likewise the occurrence of acid-fast forms of bacteria reported to exist as a transient phenomenon early in the cultures of these tissues was not confirmed.

Avian tubercle bacilli detectable by the methods used were apparently not present in the tissues of the 15 patients with Hodgkin's disease.

# THE PROLIFERATIVE REACTION OF GUINEA-PIG SKIN TO SULPHYDRYL AND ITS RELATION TO NEOPLASIA

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AND

ETHEL RAHE HANKELE, M.D.

PHILADELPHIA

Experiments for another purpose involved the application of sulphhydryl compounds to the skin of guinea-pigs. Since no results of a study of the skin of guinea-pigs after application of these compounds have been published, we record the results of our study.

## PROCEDURE

Benzyl mercaptan in a concentration of 5 per cent in alcohol was applied three times weekly for two weeks to the midscapular region of twelve guinea-pigs. Sections of the skin were removed at weekly intervals. The *p*-thiocresol in a concentration of 0.5 per cent in hydrous wool fat was applied to the same animals three times weekly. The hair over the region of application was kept closely cropped. Sections of skin were removed, fixed in Sousa's solution,<sup>1</sup> embedded in paraffin, cut at 3 microns, and stained with hematoxylin and eosin and with iron-hematoxylin. Sections were taken at intervals of two or three weeks over a period of three months. As controls, the skins of untreated guinea-pigs were used.

## RESULTS

As in rats and mice<sup>1a</sup> so in guinea-pigs the skin becomes palpably thickened from ten days to two weeks onward.

The normal skin of guinea-pigs differs from that of mice in the direction of slightly more differentiation and organization. The epithelium is from 2 to 4 cells thick. There is no special regularity except in spots. There is no clear, unbroken basement membrane, and few local groups of cells with protoplasmic bridges are present.

As early as after the second application of benzyl mercaptan, changes are recognizable. Since they are identical with those occurring

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From the Lankenau Hospital Research Institute.

This investigation was aided by a grant from a group of trustees of the Lankenau Hospital.

1. Sousa's solution is made as follows: Boil together distilled water, 700 cc., mercuric chloride, 45 Gm., and sodium chloride, 5 Gm. When cool, add 20 per cent trichloroacetic acid, 100 cc., a diluted solution of formaldehyde U. S. P. (1:40), 200 cc., and glacial acetic acid, 25 cc.

1a. Reimann, S. P.: *Protoplasma* 10:82, 1930.

in rat and mouse skins, which were completely described in previous communications,<sup>2</sup> merely a brief résumé will be given.

A clear, unbroken basement membrane appears. The cells of the basal layer become oriented vertically and are present as a very definite layer. Toward the surface from these there appears a definite layer of

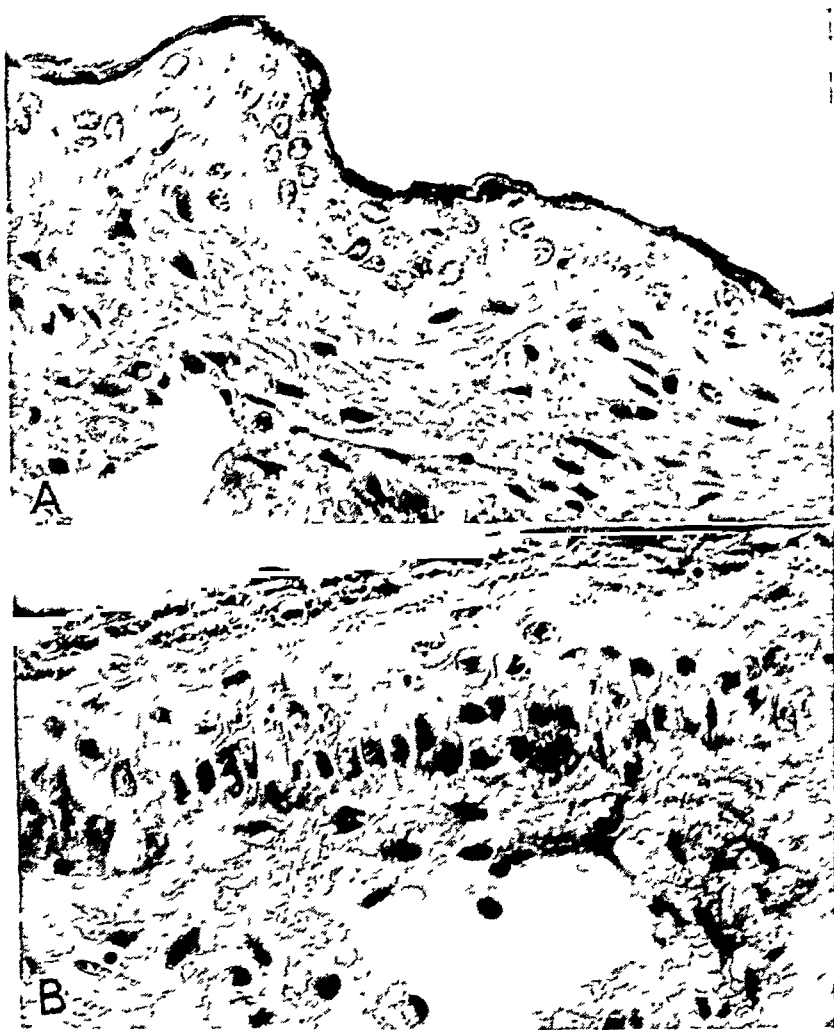


Fig. 1.—*A*, control, untreated skin of guinea-pig;  $\times 370$ . *B*, skin of guinea-pig after 5 per cent benzyl mercaptan in alcohol had been applied three times weekly for two weeks, then 0.5 per cent *p*-thiocresol in hydrous wool fat three times weekly for three weeks;  $\times 370$ .

spinous cells, and beyond them a definite layer of pigmented cells. Search of the untreated skin resulted in the discovery of two mitoses over a length of about 2 cm. Over the same length in the treated skin there were found eight, ten, eleven, nine and fifteen mitoses in as many sec-

2. Hammett, F. S. · *Protoplasma* 13:331, 1931.

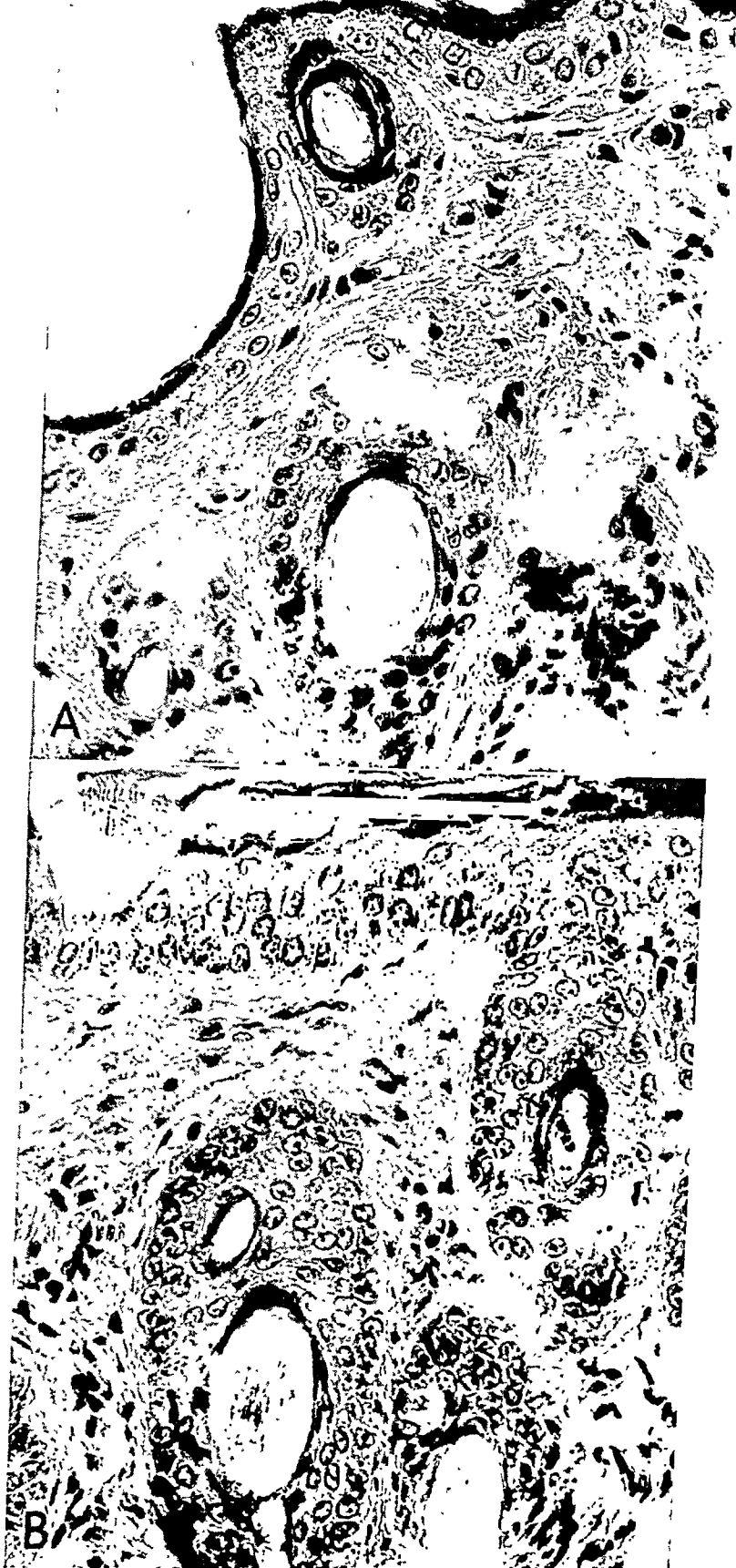


Fig. 2.—*A*, control, untreated skin of guinea-pig to show hair follicles;  $\times 370$ .  
*B*, skin of guinea-pig after 5 per cent benzyl mercaptan in alcohol had been applied three times weekly for two weeks;  $\times 370$ .

tions over a length of about 2 cm. The mitoses, whether in treated or untreated skin, were all in the basal layer.

These changes, as stated, are apparent at the end of two or three applications. At the end of from twelve to fifteen applications they apparently reach their height and remain in about the same degree for the remainder of the three months' experimental period.

#### COMMENT

The development of any organism, organ or tissue which is accomplished by the multiplication of cells occurs by the route of three separate processes occurring in these cells, namely, multiplication, differentiation and organization. These processes are separate and distinct manifestations of the potency of protoplasm. Biologically they serve different ends, and one or the other can be thrown out of "gear" separately from the others.<sup>3</sup> On the other hand, they are also related, for obviously cells cannot differentiate and organize if they are not there, i. e., they must proliferate. Conversely, if they do proliferate, they must, in order to develop normally, differentiate and organize in tune to the genus, species, individual, organ and tissue in which they are present.

In a previous communication, Hammett,<sup>2</sup> analyzing the phenomena in the skin of the rat and mouse, concluded that the more rapid the proliferation the more rapid the differentiation and organization. In addition there emerged the yet more important conclusion that increasing the rate of proliferation not only increased the rate of differentiation and organization, but also increased their degrees, from which it was concluded in relation to neoplasia<sup>4</sup> that when cells proliferate at rates greater than normal or when more cells are produced than are normal for the part, the end-result is not a neoplasm, provided that the potencies of the cells, i. e., their internal composition, remain normal. The next logical statement in regard to neoplasia is: Neoplastic cells have altered potencies of differentiation and organization.

These bald statements are taken from previous communications<sup>5</sup> in which results in additional species are recorded. To which are added the experimental results from the use not only of sulphydryl but also of a partially oxidized derivative, i. e., the sulphur equilibrium, as originally described and experiments planned by Hammett and Hammett.<sup>6</sup> A

3. Needham, J.: *Chemical Embryology*, New York, The Macmillan Company, 1931, vol. 3, p. 1650.

4. Reimann, S. P.: *Am. J. Cancer* **15**:2149, 1931.

5. Reimann,<sup>4</sup> (a) Hammett, F. S., and Hammett, D. W.: *Protoplasma* **17**: 321, 1932. (b) Reimann, S. P.: *Arch. Path.* **15**:675, 1933.

6. Hammett, F. S., and Hammett, D. W.: *Protoplasma* **15**:59, 1932; **16**:253, 1932; **19**:161, 1933. Hammett, F. S.: *ibid.* **19**:510, 1933.



tentative definition of a neoplasm was attempted on the basis of these results.<sup>5b</sup>

Some authors call this internal change of potency of neoplastic cells "somatic mutation," with which we are in agreement if, and provided that, a strict definition is given of what is meant by "somatic mutation." Otherwise biologists must accept that tar, dibenzanthracene, indeed all of the chronic irritants in general which cause or are followed by cancer are producers of mutation. This they may not be willing to do (as yet, if ever) in view of the difficulties experienced in producing mutations (acceptable to them) by temperature changes, etc., until Muller succeeded by means of the x-rays.<sup>8</sup>

This being the case, we shall continue to say that neoplastic cells have altered potencies of differentiation and organization until later agreement is reached as to definitions. Also, for the reasons given in a previous paper, which are confirmed by the results in another animal, described in this paper, we shall continue to define a neoplasm as follows: *It is a mass of cells which arise from and continue to proliferate within an organism as a result of and in direct proportion to their degree of internal qualitative differences from the other cells of the organism with respect to the potencies of differentiation and organization particularly.*<sup>5b</sup>

#### SUMMARY AND CONCLUSIONS

Another animal, namely the guinea-pig, has been added to the growing list of organisms which have been proved responsive in their rates of cell division to the sulphydryl group. As in rats and mice, when the sulphydryl group is applied to the skin of guinea-pigs there results an increase in the rate of cell division followed secondarily by an increase in the degree of cell differentiation and organization. The same implications and conclusions both in regard to general biologic principles and in regard to neoplasia are again justified from the material in these animals. The reader is referred to these papers for further details.

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7. Bauer, K. H.: *Mutationstheorie der Geschwulst-Entstehung*, Berlin, Julius Springer, 1928. Curtis, M. R.; Dunning, W. F., and Bullock, F. D.: *Science* **77**:175, 1933; *Am. J. Cancer* **17**:894, 1933. Kostoff, D.: *Protoplasma* **20**:440, 1933; Editorial, *J. A. M. A.* **102**:214, 1934.

8. Hanson, F. B.: *Physiol. Rev.* **13**:466, 1933.

# METAMORPHOSIS OF METASTRONGYLUS LARVAE AND MESENTERIC LYMPH GLANDS

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SAN FRANCISCO

Lymph glands have generally been considered to be strongholds against infections of any kind. No parasite was known to breed exclusively in these glands at any point of its life cycle. Recently it has been found, however, that this theory does not apply to *Metastrongylus*.<sup>1a, b</sup> Its metamorphosis from larvae of the infestive stage into sexual larvae takes place exclusively in the mesenteric lymph glands.

This invasion is, of course, destructive to the involved glands in varying degrees according to the numbers of migrating parasites. The cases here presented are the result of extremely heavy infestations under the experimental conditions previously described.<sup>1a</sup> A similar behavior has been found in other lungworms, as for instance in *Dictyocaulus* of sheep.<sup>1c</sup> *Metastrongylus*, however, was selected for these studies owing to the peculiar structure of the lymphatic glands of swine which enables the worker to locate the migrating larvae in a manner not possible in the glands of sheep or any other domestic animal. The trabeculae in the lymph glands of swine are highly developed, forming extensive layers of connective tissue between the follicles. Furthermore, they enclose broad lymph vessels which are discharged into the lymph sinus,<sup>2</sup> a fact essential in the course of the present investigation.

Since it was found that the infestive stage of *Metastrongylus* develops in an intermediate host,<sup>3</sup> it was possible to approach a solution of the much discussed problem of the development of lungworms in the vertebrate host.

Histologic studies soon revealed lesions, connected with the migrating nematodes, on the wall of the intestine. Inconspicuous infiltrations of eosinophilic blood cells have been encountered in the stroma of the villi, in the mucosa, in the submucosa, rarely in the muscularis around vessels and even in the subserosa. They were, however, more numerous

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From the Hooper Foundation for Medical Research, University of California.

1. (a) Hobmaier, A., and Hobmaier, M.: *München. tierärztl. Wchnschr.* **80**:365, 1929; (b) **80**:433, 1929; (c) **80**:621, 1929.

2. Trautmann, A.: *Ztschr. f. Anat. u. Entwicklungsgesch.* **78**:733, 1926.

3. Hobmaier, A., and Hobmaier, M.; <sup>1a</sup> von Schuckmann, W., and Zunker, M.: *Ztschr. f. Infektionskr.* **38**:353, 1930. Schwartz, B., and Alicata, J. E.: *Parasitology* **18**:21, 1931.

in the region of the colon and cecum than in other parts of the intestine. Scattered cells of that type or aggregations of them have been observed in those regions. Furthermore, the local eosinophilic infiltration was strongly marked on the mesentery in the vicinity of lymphatic vessels between the intestine and lymph glands.

Practically every lymph gland of the intestine has been found invaded by the nematodes, owing to the extremely large numbers of larvae administered to the animals. The larvae, however, varied greatly in number in different sections of the intestine. They were most frequently found in the lymph glands of the colon and cecum and in those of their vicinity. Their numbers decreased steadily toward the rectum and toward the stomach. No larvae could be detected in any lymph gland not directly connected with the digestive tract.

So far as pathologic changes are concerned, two phases may be distinguished. The acute stage lasts about six days, beginning with the invasion of the glands by the migrating larvae and ending with their emigration. The chronic phase or stage of reparation, on the other hand, is to be noted following their emigration.

During the first period the involved glands appear enlarged. They present a spherical or finger-like shape. The capsule is extended. No hemorrhages of any significance are visible. On sections of the glands the tissue is seen protruding and rich in fluid.

Histologic investigations showed the larvae accumulated in the lymphatics of the trabeculae. It seems that ordinarily the larvae of *Metastrongylus* do not enter the marginal sinus before they have grown into the sexual stages. The pictures favor the belief that most of the infestive larvae are arrested in the lymphatics of the trabeculae where the larger lymphatics lose width near the marginal sinus. In other words, an embolization takes place. As soon, however, as the larvae have grown from the infestive into the sexual stages, an exsheathment follows, and they continue their way into the marginal sinus and from there pass in the prescribed way of the lymphatics until they become finally arrested for a second time by embolization in the capillaries of the pulmonary artery. It need hardly be mentioned that studies on structural features of the larvae require fresh preparations; meanwhile, histologic investigations are required in topographic studies concerning the exact localizations of the larvae. Feeding of a large number of larvae makes it easy to recover many of them in lymphatics of the trabeculae. The disadvantage of this method, however, is that the anatomic structure of the glands may become endangered and as a consequence at least some of the larvae may take an abnormal route of migration.

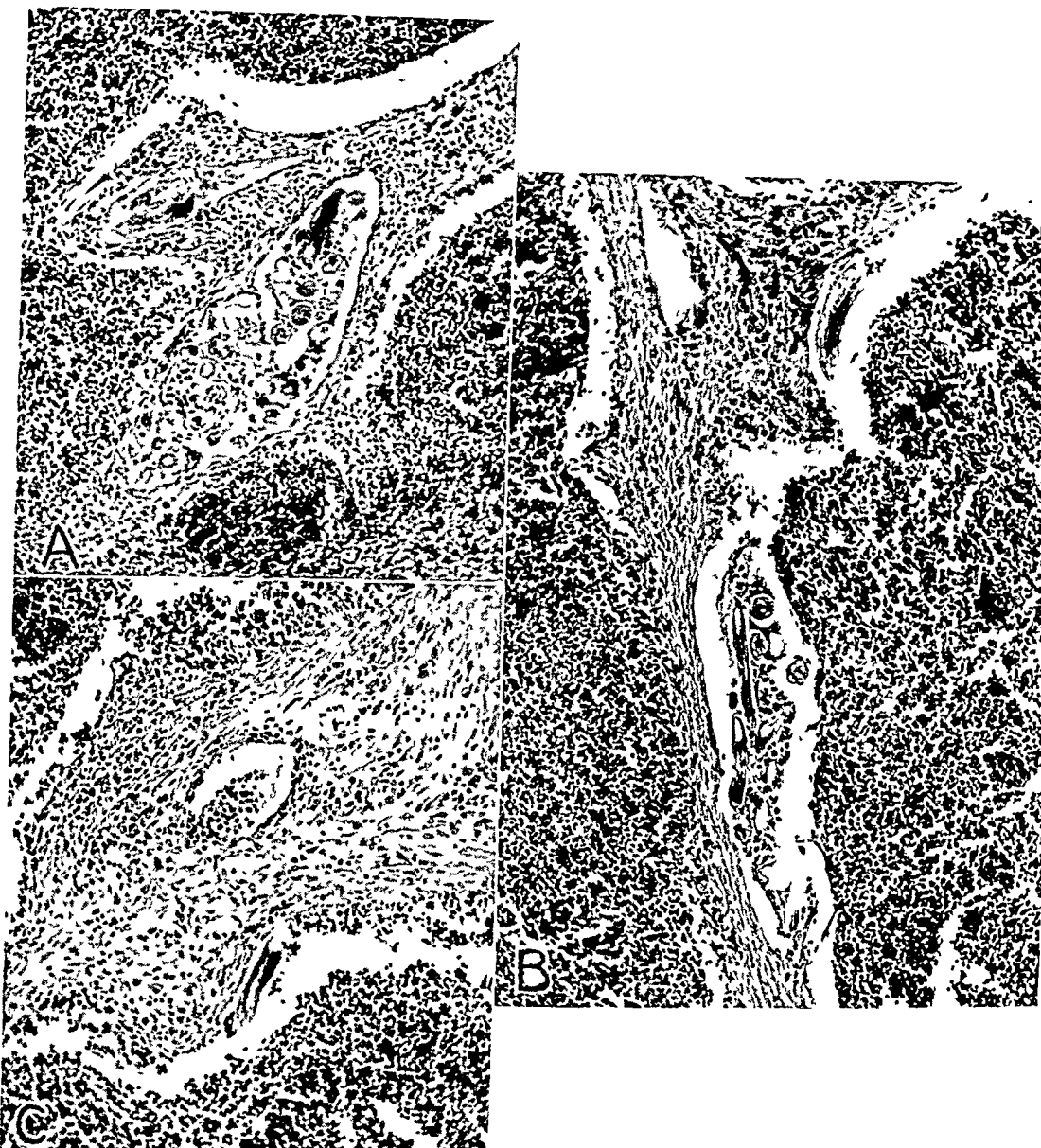
The lumens of the invaded lymphatic vessels appear distended. The larvae may be found curled up in the vessels, varying greatly in numbers (fig. A). No peculiar reaction takes place at this time so far as the walls of these vessels are concerned. Only a few lymphocytes and some eosinophils may be found between and near the larvae. A paucity of coagulated plasma may be seen occasionally. Some of the larvae show different stages of molting. The trabeculae themselves appear enlarged. Their normal structure is supplanted by a reticulum of angioblasts and fibroblasts presenting the picture of a young granulating connective tissue. Eosinophilia is present throughout the trabeculae. Most frequently the lymph vessels are surrounded by these cells. Occasionally ecdysis may be observed on larvae enclosed in lymph vessels bordering the lymph sinus. It seems that the wall of the vessel attenuated by the pressure of the growing larva bursts under the stronger movements of the exsheathed larva, dismissing it into the marginal sinus (fig. B).

No eosinophilia is seen in the lymph nodes. Their center is often poor in lymphocytes. Their peripheral region borders without sharp lines the marginal sinus. The latter presents the usual picture of sinusal catarrh. Its reticulum is completely filled with a superfluity of lymphocytes and eosinophils. In several places sections of well developed (sexual) larvae may be found. Occasionally small foci, consisting of the remnants of a nematode larva surrounded by a mass of eosinophils and lymphocytes, have been encountered.

During the second phase, the period after the larvae have left the lymph glands, a reparation of the disintegrated tissues may be seen in progress. Macroscopically the glands are still larger than usual. However, they now appear harder than normal glands and more strongly attached to the surrounding tissue.

The microscopic pictures of the affected glands vary greatly in different sections. In some places the normal structure is completely lost; in other parts there is little alteration beside the eosinophilic infiltration. The great variety of the microscopic aspect due to the migrating larvae may be easily understood, considering the irritation of the connective tissue with its vessels which are present in the trabeculae and in the stroma of the glands. As often seen in pathologic changes, the response to the injury is not confined to a limited and what seems to be an adequate reparation, but an excessive granulation precedes the definite repair. An abundance of vascularization generally produces an abundance of granulation. These conditions are fulfilled to a high degree in the lymph glands. Consequently the reaction to trauma is strong in these glands.

The angioblasts and the fibroblasts of the trabeculae grow into the marginal sinus, disregarding the anatomic border lines. The lymphatic



Mesenteric lymph gland showing *Metastrongylus* larvae. *A*, a mesenteric lymph gland. Two lymphatics are visible in the trabecula. The lymphatic vessel above contains a longitudinal section of a *Metastrongylus* larva. In addition to that, a small amount of coagulated plasma is visible. The lymphatic vessel below shows extreme dilatation. The trabecula itself is attenuated in its vicinity. The wall of the vessel is ready to open into the marginal sinus. The lumen of the vessel is filled with segments of the larvae embedded in coagulated plasma intermingled with eosinophils. *B*, the lower part of the trabecula is attenuated. A number of longitudinal and transverse sections of *Metastrongylus* larvae may be seen in the lymphatic vessel. The wall bordering the marginal sinus is ruptured, and the larvae are about to invade this region. In the upper right corner of the marginal sinus a longitudinal section of a sexual larva already liberated is visible. *C*, enlarged trabecula consisting chiefly of angioblasts and fibroblasts intermingled with eosinophils. The wall of the lymphatic vessel in its middle shows an eosinophilic infiltration and the early repair of the wall following the emigration of the larvae. A longitudinal section of a newly liberated sexual larva of *Metastrongylus* may be seen in the marginal sinus below.

cells, on the other hand, invade the spaces left between the granulating tissue. The lymph nodes stimulated to high activity discharge large quantities of lymphocytes into the environment, thus overflowing the region of the marginal sinus and disarranging the entire structure of the lymphatic gland. Under these circumstances the trabeculae are sometimes reduced to a few angioblasts and fibroblasts; sometimes they appear considerably enlarged and merge indistinctly into the nearby tissues.

In other parts of the glands the normal structure may be easily recognized. The trabeculae may be seen rebuilding their fibrous structure. The border lines between lymph nodes and trabeculae may be clearly marked. The lymph sinus may present the normal appearance. In other cases, many capillaries are seen crossing this interspace, accompanied in places by strains of connective tissue. A paucity of lymphocytes is apparent only in the meshes left between the capillaries and the connective tissue. It is noteworthy that the eosinophilia still endures in the course of these changes.

Small quantities of coagulated plasma may sometimes be found in lymphatic vessels invaded by larvae. These small thrombi finally become organized with a limited number of lymphocytes, white blood cells and eosinophils. They seemingly immigrate through the wall of the affected vessels involving this part of the wall in the process of reparation, as shown in figure C.

It is generally known that young swine are more readily affected by lungworm disease than older ones. The normal anatomic and the pathologic changes in the tissues of the host during life, and especially the changes taking place in mesenteric lymphatic glands, may be at least partially responsible for the higher resistance, rather than biologic processes of immunity.

Since the discovery of Stewart concerning the migration of *Ascaris* in blood vessels it has been suspected that larvae of lungworms may act in a similar way. In accordance with that theory the migrating larvae would be found passing through the liver. The tissues of the lungs, on the other hand, would show, besides exsheathed larvae of the infestive stage, ensheathed sexual larvae during the first days after infection.

No macroscopic changes could be observed in the liver. Large areas of the liver have been investigated microscopically in fresh preparations, but without success. Microscopic slides of fixed material taken from about one hundred different points showed changes due to a nematode's having recently migrated in the tissue in three specimens only. The larvae themselves, already in a state of decomposition, were situated on the periphery of the lobules. A small accumulation of round cells and eosinophils surrounded the dead nematodes. The same cells were found

in the vicinity and especially in the nearby interlobular connective tissue. It was, of course, not possible to determine whether these larvae were originally a part of the larvae fed to the animal, though it is probable. If this is true, their peripheral localization appears to indicate that the larvae were already too large to pass through the capillaries of the liver.

Investigations of similar material conducted one and two months later still showed a local eosinophilic infiltration, where larvae had previously perished. No production of fibrous tissue in any considerable amount had taken place. No macroscopic changes were seen. There is apparently no connection between lungworm disease and parasitic interstitial hepatitis in swine as claimed by different workers.

An extended search in the lungs during the time of immigration resulted in the detection of a few exsheathed larvae of the infestive stage. No ensheathed larvae of the sexual forms could ever be found, but exsheathed sexual larvae were present in exceedingly large numbers. No molting larvae were found in the lungs, but it was possible to observe on fixed material a few tubercles embedded in the region of the alveoli. They consisted of the débris of a minute nematode larva. An aggregation of round cells, eosinophils and of one or two giant cells surrounded them. These tubercles may have been the product of a biologic reaction against erratic infestive larvae, which were apparently not yet sufficiently developed to live and to grow in this environment.

#### CONCLUSION

The present study corroborates the previous statement of A. Hobmaier and myself that the infestive larvae of *Metastrongylus* must invade the mesenteric lymphatic glands of the vertebrate host to grow there into sexual larvae and that they do not enter the tributaries of the portal vein as a part of their regular life cycle.

The pathologic changes observed in the invaded lymph glands may be explained as the result of the trauma inflicted by the embolization of the larvae into the lymphatics, by the dilatation and occlusion of these vessels during the development of sexual larvae, and by the disarrangement of anatomic structures caused by their emigration.

# AN ANALYSIS OF CORONER'S STATISTICS FROM COOK COUNTY (CHICAGO), ILLINOIS

WITH A PATHOLOGIC REVIEW OF THE CAUSES OF DEATH

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## INTRODUCTION

The present study is a statistical survey of all deaths investigated and passed on by the coroner's staff of Cook County, Ill., within the four years of a single term of office, from November, 1928, to November, 1932.

The question of the coroner system versus the medical examiner system is outside the scope of this study, but significant references follow.<sup>1</sup>

This period was taken as representing exceptional conditions for efficient functioning of the coroner's medical personnel. It was felt that under such conditions the general trend of the statistical data of the coroner's system is similar to that of a more ideal system.

The coroner's physicians were chosen from a group qualified by previous pathologic training. They were selected by an advisory board appointed by the coroner, composed of professors of pathology of the four major medical schools of Chicago. This method of selection secured physicians who were not only adequately trained but free from political influence and demands. Furthermore, the wide experience of the advisory board was made freely available in the organization of field work and in the solution of pathologic and medicolegal problems.

The importance of an efficient unit of trained examining physicians as an aid to the state in deaths involving medicolegal questions needs no further statement here. However, it is felt that there is need of statistical analysis of the mass of death records piled up by the coroner's service. In such analysis various broad relationships are involved since the coroner's work integrates with numerous other departments and agencies of the body politic. Some of these relationships will be briefly mentioned here as indicating the scope of the work.

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From the Department of Pathology of Northwestern University Medical School.

1. Schultz, O. T., and Morgan, E. M.: The Coroner and the Medical Examiner, National Research Council Bulletin No. 64, Washington, D. C., 1928.  
Martland, H. S.: Proc. Inst. Med. Chicago 9:261, 1933.



The primary growth of the coroner's function related to questions of criminal acts in individual deaths, and such investigations are still perhaps the most widely known of his activities. In this capacity the coroner's physician is required to furnish to the state's attorney all immediate material evidence of crime, as well as an opinion as to the determining cause of death and the associated pathologico-anatomic findings in the dead body. Present day trends of crime soon bring this activity into relation with gangism, robbery and criminal abortion, to mention some of the crime ramifications.

The coroner at present reviews and certifies all deaths which are not due to so-called natural causes, and so furnishes to the health department a rapidly growing mass of statistics on deaths related to external traumas. His investigation of circumstances leading to accidental deaths of workmen gives information to the state industrial department valuable in formulating preventive safety measures and in determining compensable injuries. Public safety organizations likewise find in his work material support for measures and public education looking to the prevention of accidents. Insurance companies have a rich fund of information provided by the coroner on which to shape policies in regard to industrial injuries or transportation accidents, accidental poisonings and suicides, for example.

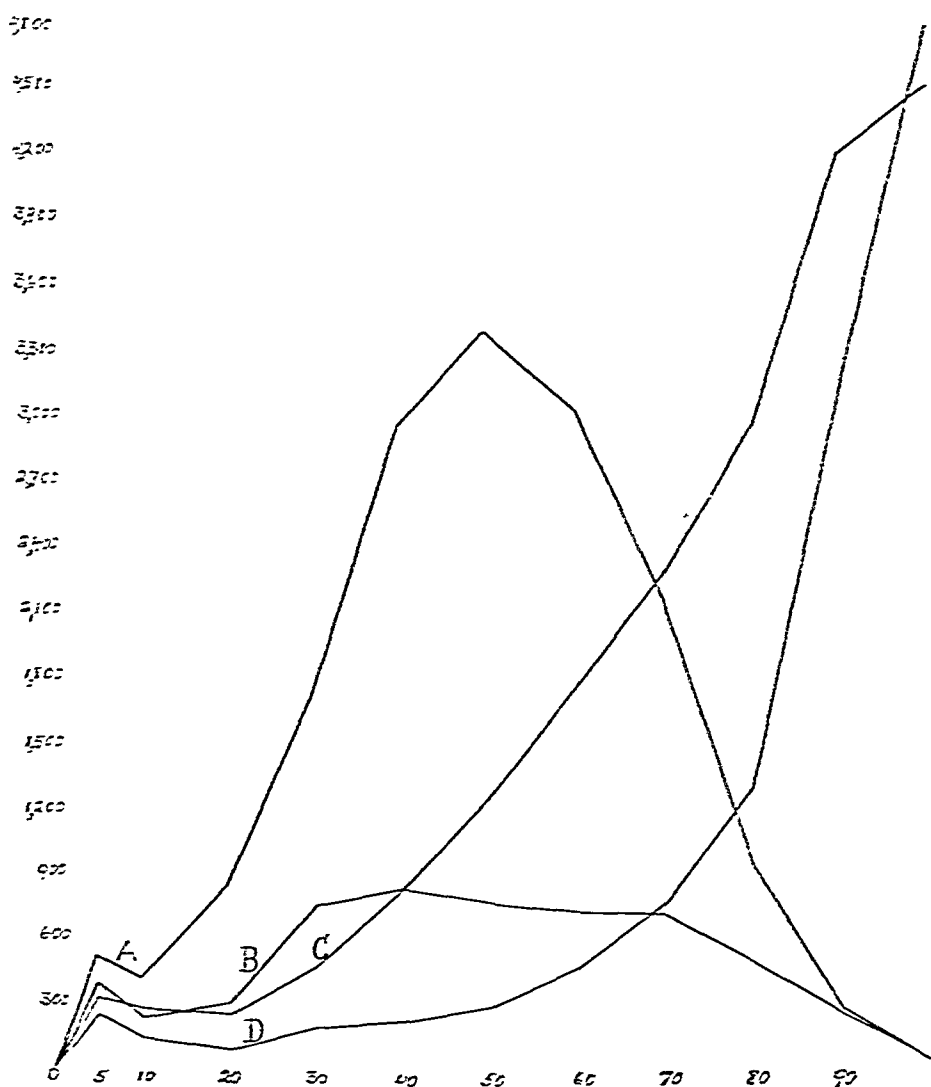
In addition, since various previously existent or intercurrent illnesses and physical disabilities may play a significant rôle in the outcome of injuries an analysis of mass data revealing the importance of these and their mode of action will be valuable to practicing physicians and to pathologists.

In all of the aforementioned relationships of the services performed by the coroner's physicians, it may be added, perhaps unnecessarily, that the value of the coroner's information depends on the type of pathologic investigation done.

#### GENERAL STATISTICAL SURVEY

The total number of deaths investigated by the coroner's physicians in the four year period, 22,206, represents 13 per cent of the total number of deaths in the area. A large proportion of them occurred within the city of Chicago, and of the city's total number of deaths they comprised 15.5 per cent. Previously the number of coroner's cases had been much higher, reaching 8,374 in 1926, for example, or 20 per cent of the total number of deaths in the county. The reduction in numbers was brought about by certain changes in the rules for acceptance of cases. Among these was the abatement of a rule requiring report to the coroner of any death occurring in a hospital within twenty-four hours after the patient's admittance. Another was the refusal to

view any case of death occurring at home if a physician had been summoned for the first time at about the time of death. If the physician would not sign the death certificate but supposed the death to be due to natural causes, the case was referred to the local registrar, under the department of health.



A and B show the total numbers respectively of males and females who died in the different age groups: C and D, the rates of death of males and females per hundred thousand of population. The age decades are indicated at the bottom.

*Sex.*—There was a total of 16,426 males and 5,425 females whose deaths were investigated, with 355 fetuses not included, making a proportion of males to females of about 3:1. The types of risks resulting in so-called coroner's deaths certainly are assumed much more frequently by males. Among these may be mentioned heavy mechanical

labor, other physical activity with exposure to injury, drunkenness, suicide and homicide.

*Age.*—Such risks among others are operative over the greater part of the life span, with a resultant increase of mortality. Beginning at the age of 10 the proportion of males to females in successive decades was roughly 3:1, 2:1, 3.5:1, 5:1, 4:1, 3:1 and 2:1. This difference was decreased at the extremes of life, as might be expected.

Deaths of children under 5 years of age numbered 925. A large number of these were due to some type of asphyxiation, such as that from smothering, from aspiration of a foreign body or from a respiratory infection. A large number were due to falls. In deaths due to falls or to accidents of transportation fracture of the skull was the common finding. Two factors are probably operative in this injury. One is the lack of protection afforded by a weak general musculature and the other is the ratio of the weight of the head to that of the body. The weight of the head is 27 per cent of that of the body at birth, decreasing to 16 per cent at 5 years of age, while in the adult it is 7 per cent. Another important cause of death in this group was burns, most frequently from hot water, coffee, tea or soup.

In the group of advanced age, with 554 deaths in those more than 80 years old, cardiovascular mishaps were responsible for the largest number of deaths. Cardiac failure on a myocardial or coronary basis was the most frequent cause, with spontaneous hemorrhage, thrombosis and embolism next. A second very frequent cause of death in the aged group was injury sustained in a fall, often accompanied by fracture of bones and quite commonly terminating in bronchopneumonia.

Fetuses numbered 355. These represented largely calls to the police who picked them up and took them to the morgue. It is very possible that this number is a wholly inadequate measure of the total number of dead fetuses. Those reported varied from very early fetuses to fully matured infants at term. Evidence of malformation or disease of the infants or membranes was quite rare. The suspicion must remain strong that an abortion carried through without medical attendance and with surreptitious disposal of the products of conception savors of criminal practice. The full-term infants gave mute evidence of all forms of taking of life and, often, attempted destruction of the body. Hemorrhage from the cut, untied cord, choking, smothering and blows on the head were the most frequent means used to kill.

Little comment is ever made on this taking of life, and it certainly offers problems difficult to solve. It is likely this difficulty which governs the lack of legal investigation into most of such cases, which are quite largely cases of either murder or manslaughter.

*Race.*—The chief racial elements found in the population are the Caucasian and the Negro. Of these the white persons made up 89 per cent and the black 11 per cent of the coroner's deaths. Only occasional deaths of persons of other races appeared. The yellow race had a list of 44 cases. The Negro population of the area is 250,000 in a total of 4,000,000, or about 6 per cent. It is seen that deaths in the Negro population appeared as coroner's cases in a somewhat higher ratio than deaths of the whites. As to location, practically all of the bodies of Negroes were found in colored districts or at the morgue.

*Length of History.*—The majority of cases showed, as expected, a short duration of time from the onset of trouble to death; in 17,401 there was a history of less than twenty-four hours. A large share of the deaths, then, were of emergency nature. Of cases with a history of from one to fourteen days there were 3,049. Of those with a history of from two to eight weeks there were 761, and of those with a history of from two to twelve months there were 427, while in 213 cases the

*Total Number of Deaths by Months*

	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
1928	789	732	...	...	...	...	...	...	...	...	...	...
1929	381	409	574	390	564	566	434	366	366	373	324	356
1930	456	418	385	401	378	433	399	429	441	425	361	363
1931	598	510	414	368	402	421	435	480	525	519	489	513
1932	...	...	499	435	528	503	502	505	527	507	451	487
Total	2,124	2,069	1,872	1,654	1,572	1,923	1,770	1,811	1,859	1,837	1,656	1,749

victim lived one year or more. Many things militate against large numbers in the later brackets, chief of which is the fact that a disorder lasting any considerable length of time is usually entrusted to a physician's care and thus noninjury cases are weeded out. The cases with longer history were usually claimed to have some association with injury, although this association often became less definite with time.

*Time of Year.*—This question was reviewed to find if any seasonal influence might produce a marked variation of the number of cases. The total numbers by months as given in the table show no consistent influence exerted by the time of year. The large number of factors which produce coroner's deaths seem to equalize the total numbers of cases so that any seasonal difference is absorbed.

#### OPERATION OF CORONER SYSTEM

*Places of Investigation.*—Ordinarily only certain criminal cases or cases suspected of being criminal require the viewing of the body by a coroner's physician before it is moved. Quite frequently in such cases, also, when death is not instantaneous the victim is rushed by

police ambulance to a hospital. If the patient is dead on arrival or before admittance to the hospital, the body is taken to the nearest undertaker or to any other requested by a relative. Undertakers also receive bodies in cases of sudden death on the street or in public buildings. In addition, on permission from the coroner's office a large number of bodies are removed by undertakers from hospitals and homes before they are seen by the coroner's physician.

**Undertakers' Morgues:** The majority of cases, then, are investigated at undertakers' morgues. The difficulties inherent in this situation have been discussed by others.<sup>2</sup> Of the total of 22,206 cases for the period, 12,927, or 58.2 per cent, were examined in undertakers' morgues. Of these, 2,414 were given pathologic investigation, the number of autopsies constituting 39.4 per cent of the total number of autopsies. Autopsy was made in 18.7 per cent of cases investigated at undertakers' morgues.

**Coroner's Morgue:** The coroner's morgue receives bodies from the county hospital in coroner's cases in which the victims were admitted before death, and from the hospital at the city jail. Also unknown and unclaimed bodies from various other places are received. These cases are investigated entirely by one coroner's physician assigned to work in the morgue for a given period.

The total number of cases handled at the county morgue was 5,240. This represents nearly one fourth of the total number of coroner's cases. In 2,799 of these autopsies were made, or in 53.4 per cent. Also there were brought to the morgue 355 fetuses.

The advantages inherent in such a central workshop as the county morgue are numerous. Reliable information is obtained before death in most of the cases, and often from the living patient in the hospital. The relationship of previous diseases to the present trauma as the cause of death can be better evaluated with historical and clinical data available. As to the work itself the facilities of an organized morgue are offered.

This working arrangement is reflected in the number of complete investigations, accompanied by autopsies, performed at the morgue—2,799 on 5,240 bodies as compared with 3,329 on 16,966 outside bodies. The percentage of autopsies at the morgue thus reaches 53.4 as compared with 19.6 outside.

Conditions at the county morgue, however, furnish but few of the advantages which might arise from a central pathologic institute housing a general morgue to take care of all coroner's cases, with provision for pathologic or other medical scientific research under the direction of a permanent full-time pathologist. At present the work at the morgue is necessarily limited to a routine handling of individual cases as they are presented.

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2. Chandler, A. H.: Boston M. & S. J. **194**:728. 1926.

Hospitals: The remainder of the cases, numbering 3,684, or 16.6 per cent of the total, were taken care of mostly in scattered hospitals with a few at private homes. Among these cases there were 915 autopsies, the percentage being 24.8. This work is done under field conditions with many influences in action rendering investigation less satisfactory, from the standpoint of the coroner's physician, than in the morgue. While a larger proportion of this group than of that found at the county hospital is of the accident-with-sudden-death type, this larger proportion is not enough to account for the fact that the percentage of autopsies is less than half that at the county morgue.

*Disposal of Cases.*—Rejections: It is left somewhat to the discretion of the coroner's physician as to what disposal is made of the cases assigned to him. If he finds nothing in the circumstances to warrant proceeding further with a given case it may be rejected and no record of the case filed. With intelligent selection at the central office these cases are few.

External Examinations: The larger proportion of cases receive only "viewing," or external examination of the body and sometimes the surroundings, clothing or other externals to establish identity and perhaps evidences of external injury. Such external physical findings are considered together with the history in determining the cause of death. These cases numbered 16,078, or 72.4 per cent of the total.

Much objection may be raised against the policy involved in certifying causes of death without certainty as to the final pathologic condition. Any one who has been engaged in medical work on coroner's cases recognizes the type of apparently simple case of death from natural causes which is much complicated later by the uncovering of information which should have been proved by autopsy in the beginning. It is stated legally to be the duty of the coroner or his deputy to take charge of the body assigned to him by law and by his investigations to determine the cause of death. The intent of the law seems clearly shown, but its operation is vitiated by certain interpretations and restrictions, such as that limiting autopsy to cases of death from known or strongly supposed external causes.

Autopsies: A full autopsy would appear to be the only means of certifying the cause of death, certainly in most coroner's cases. One might exclude from this requirement cases which have had competent clinical investigation with a satisfactory determined pathologic cause of death. Even here, however, there may be interpretative errors clinically<sup>3</sup> with the best of conditions and experience. When this basic requirement for accuracy is sacrificed politically to various interested parties the medical certification of cause of death is stultified to that extent.

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3. Cabot, R. C.: J. A. M. A. 59:2295, 1912.

The cases in which autopsies were made, 6,128, comprised 27.9 per cent of the total number of cases. Of the autopsies, 4,389 were held in cases of death from external trauma, or in 26.4 per cent of such deaths, and 1,739 in cases of death from disease, or in 30.9 per cent of such deaths.

**Certifications:** Every case investigated must be either signed out by death certificate or referred to the central office for an inquest. The investigative function of the physician ends with determining the cause and as much as possible of the manner of death. If this seems to involve no legal liability, the coroner's physician is allowed to dispose of the case directly by signing a death certificate. In the period under consideration 5,628 cases, or about one fourth of the total number, were so handled.

Most of the cases with natural causes of death fall into this category, although if any suspicious circumstance exists or is declared concerning such a cause of death, inquest may be demanded.

**Inquests:** All other cases, 16,223, were completed by coroner's inquest. In such instances, the final determinative circumstances of death, if found, are passed on by a coroner's jury, whose verdict may close the case unless further legal action is indicated by some agency other than the coroner's office.

The latitude allowed the physician in choosing between the certificate and the inquest in the disposal of a case is determined by the policy of the coroner in office. It certainly would be much more economical as well as helpful in securing uniformity of policy if the type of case demanding the deliberations of a coroner's jury were more strictly defined.

#### INJURIES AS CAUSES OF DEATH

All causes of death in coroner's cases have been considered under the general headings of injury and sickness. When injury and sickness were intercurrent, the primary cause of death as indicated by the report of the coroner's physician has been followed.

The general rule is that any death preceded by an external injury shall be reported to the coroner's office. In application this includes any death following a recent injury of serious extent, and any death following an old injury if there is a supposed causal relationship. The trauma may arise in any manner or from any agency.

*Accidents.*—Accidental deaths naturally make up the larger share of the deaths that concern the coroner, and their number must increase with the complexity of a mechanized civilization. For convenience they are divided into three groups.

1. *Industrial accidents* resulting in death reported to the coroner numbered 610 for the four years. This remarkably low average of

153 deaths per annum reflects the modern evolution of safety devices and of "safety consciousness" in both employer and worker. The proportion of men to women killed was that of 100:1, which also indicates the average employment ratio of the sexes in hazardous occupations.

The coroner's usefulness in industrial cases depends on an immediate impartial review of the manner of the accident and a determination of the complete cause of death by the coroner's physician. The determined circumstances of death furnish data for safety recommendations, as well as information which may or may not support claims of dependents for compensation. Closer cooperation between the department of labor and the coroner's office than now exists seems therefore to be desirable in the study of the problems offered in industrial deaths.

Coroner's physicians at present may be employed by either party in a suit before the state compensation board as expert witnesses in the coroner's cases which have been assigned to them since, as county employees, they owe no special duty to the state compensation board. Further, the compensation board has no power of determining the cause of death in any case at issue other than by records or witnesses available. If industrial deaths were regarded as deaths in which autopsy is mandatory, complete data as to the cause of death could be had for record, thus wiping out frequently long argument and pure speculation.

2. Deaths from accidents of transportation numbered 10,768, or very nearly one half of all the coroner's deaths. The average of 2,692 deaths each year was 8 per cent of all deaths from whatever cause. This tremendous sacrifice of human life has been the subject of much writing and of some serious thinking. However, during the stage of development of rapid road transportation the toll of lives increased. It seems to have reached a peak and for some time has remained nearly stationary. The figures for the four consecutive years in question are 2,627, 2,794, 2,826 and 2,521. However, if the road development and present mechanical safety devices are again rendered inefficient by demands for increased speed the numbers of deaths may again increase.

The automobile gave by far the larger number of deaths, with 7,653 deaths directly charged to it and 745 more charged to it and to some other agency jointly. Railroads claimed 1,609 lives, but very few of passengers; quite a few of these lives were those of railway employees, but the greater proportion were exacted of persons not included in either of these groups. Street cars killed directly 393. Various other means of transportation added a few more deaths to the list.

3. In the group of deaths from miscellaneous accidents only those causes are considered here which piled up considerable numbers of fatalities.



Burns were responsible for 640 deaths, of which 409 occurred in adults and 231 in children. As noted before of children's deaths, those of infants were caused mostly by their being burned by hot liquids, and those of children 5 years of age or older, mostly by fire igniting their clothing. Fatal burns of adults were generally by fire. Several deaths occurred from igniting the bedclothes with cigarets but usually in persons who were known to be or who probably were addicted to overconsumption of alcohol. The history of alcoholism was so general that it would appear to be a definite hazard for a drunken cigaret habitué to be put to bed with cigarets and matches within reach.

Of asphyxial deaths (aside from suicides), numbering 470, a large share was caused by illuminating gas at home. A portion of the public does not yet realize the possible danger of a gas-burner attached by a temporary, often leaky connection such as a rubber hose, or the danger of a gas-heater, especially a stove, in a small unventilated room. Quite a few deaths from gas occurred as a result of going to sleep in a closed room with water or other liquid heating over a gas flame. When the liquid boiled over the fire was extinguished but the gas continued to escape.

Illuminating gas is a highly complex mixture the properties of which are imperfectly understood by the average consumer. This ignorance should be assumed to be in effect by the supply companies and a warning as to the common dangerous errors in the use of gas posted in every gas user's home. The supply companies should be required to post such warnings.

It is probable that many suicides by gas are called accidental because of lack of proof or other reasons. This is especially true of suicides by motor exhaust gas, which ran second among causes of deaths from accidental asphyxia.

Deaths from drowning, aside from suicides, are divided into two groups: those determined to be accidental, 368, and those in which the circumstances remained undetermined, 345, or a total of 713.

It is significant, from a legal standpoint, that in nearly half of the cases of drowning the circumstances remained undetermined. It is true that they offer some of the most puzzling of medicolegal problems. Many drowned persons are picked up floating after an indefinite time in the water, often in an advanced state of decomposition and commonly devoid of ordinary identification marks. Ruling out foul play may be difficult<sup>4</sup> and decision as to accident or suicide impossible. A very accurate chemical method has been devised<sup>5</sup> to determine whether death occurred by drowning or not. In this regard, the question of

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4. Buetz, G.: *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:557, 1932.

5. Gettler, A. O.: *J. A. M. A.* **77**:1650, 1921.

mode of death in drowning arises again. This was investigated recently in animals,<sup>6</sup> with the finding that considerable amounts of water entered the lungs. In human beings, with different types of reflexes, however, death in drowning may not always result from the entry of water into the lungs.

Accidental falls causing death occurred in almost every conceivable manner, some of them being rather trivial in nature. The most of them were included in falls on the floor, 178 cases, and falls from high places, 433 cases.

The falls on the floor included all simple falls from the standing or sitting posture to the level of the feet. Death was caused from fractured skull or ruptured viscus, or late from pulmonary embolism or bronchopneumonia or other infections. The last named condition resulted in death in old people, for whom especially falls have much danger. Generally death from a minor fall presupposed some added hazardous factor such as age, weakness, sickness, drunkenness or striking against some projecting solid body.

The falls from high places again bring up the question of possible suicide in many instances. This group probably is larger than it should be, since all unproved cases, even though the question as to possible suicide may have been present, were classed as accidental.

Falls from hospital windows were responsible for 14 deaths. While this included the inevitable number of disoriented persons who eluded all restrictions, yet there was a marked proportion of patients with incurable maladies, such as malignant tumor, who chose this way out of their difficulties. Since the hospital assumes a certain responsibility for its patients, and since there is no way of knowing which patient may be seized by an impulse to hurl himself out of the window, it would seem much safer to provide some protective device at the windows. Yet the new hospitals which are built consistently ignore this feature of safety for the patients and freedom from legal attack for themselves.

Rupture of a single viscus from external trauma, aside from that caused by a penetrating wound, was found as the cause in 116 deaths. Most of these occurred from violent force such as results from falls or from the impact of a car or other heavy object, and so were generally associated with more or less shock. Several instances were found, however, of rupture of the liver or of the spleen in otherwise relatively minor accidents. Such a complication may be discovered easily if the patient is under supervision in a hospital, but most of these who died from this cause did not have such favorable care.

There were 6 instances of rupture of the small intestine, and in 2 of these the intestine was completely severed. A case which I observed may

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6. Karpovich, P. V.: *Arch. Path.* **15**:828, 1933.

be cited as an example. A young man, struck in the back by an automobile and knocked down, walked several city blocks to his home, complaining only of pain in the back. Twelve hours later he suffered from abdominal pain, which was treated by a heavy dose of magnesium sulphate. This was repeated and the man died at home on the third day with no further care. Autopsy revealed generalized peritonitis. The ileum was completely severed at its midportion, as sharply as if cut by a knife. No trauma was found on dissecting the muscles of the back.

*Suicides.*—The tendency toward suicide is one of serious social concern in the present phase of evolution. The number of lives lost is considerable, but this is outweighed by the social-economic significance of the suicidal act itself. Each misfit or failure who sentences himself to death is thus voicing judgment against intolerable living conditions. So one might expect any sudden marked increase in the general stress of living to take its toll of those unable to adjust themselves to its demands. The association of suicides with economic crises is the most frequently cited illustration of this phenomenon. Suicides for this four year period numbered 2,723, which represents an average rate of 17 per hundred thousand. In the four years the rates ran successively 15.8, 17.3, 18.3 and 16.7.

On analysis of the cases of suicide there are found 2,138 males and 585 females, a proportion of almost 4:1, somewhat higher than most of the rates given.<sup>7a</sup> The white race accounted for almost all of the suicides, 2,607 cases; only 102 cases were contributed by the colored race. The remaining 14 cases occurred in various other races. This disproportionately high number of suicides of whites is in line with other published reports.<sup>7b</sup> The colored race seems to react very little to the depressing influences that promote suicidal attempts. As to the social condition of those who committed suicide, 1,542 were married, giving a suicide rate of 21.7; 608 were single, giving a rate of 15.9, and the remaining 573 were widowed or divorced, the rate reaching the high figure of 54.2. A recent report from New York City<sup>8</sup> likewise indicates rates for the latter class of persons much higher than those for single and married persons.

Of the methods used for committing suicide, shooting accounted for 802, considerably more than can be ascribed to any other means of self-destruction. Poisoning with 496 cases, hanging and strangulation with 494 and asphyxiation with 461 each accounted for about the same number. At a considerably lower level of numbers were those

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7. Hoffman, F. L.: *Suicide Problems*, Newark, N. J., Prudential Insurance Company of America, 1927, (a) p. 249, (b) p. 15.

8. Hoffman, F. L.: *Spectator*, June 8, 1933.

who ended their lives by jumping from high places, 168, by cutting or stabbing themselves, 158, and by drowning, 116.

It is to be noted that the ratios of deaths by these various means are considerably different from those reported in late years from New York City.<sup>9</sup> In these reports illuminating gas has come to the fore as a ready means of self-destruction. In the report for 1931, cases in which gas was used numbered more than double those of any other form of suicide. Jumping from high places accounted for the second highest number, with hanging, shooting and poisoning following in the order given.

It is probable that a good proportion of those who attempt suicide use any means ready at hand for accomplishing the purpose. In Chicago the acquiring of firearms is easy and possessing them is not threatened with any serious consequences, so that they may be found rather commonly in homes. Whether a law such as the Sullivan law of New York would lessen the total number of suicides or would only divert those achieved by shooting into some other category remains questionable.

Jumping from high places has not increased so rapidly in this city as in New York City. It is given undue stress in the daily press because of its spectacular nature and because it has claimed some rather prominent victims. Possibly press emphasis may increase the numbers in the future.

Asphyxiation by gas, which is far ahead in numbers in New York, takes only fourth place in Cook County. I have found illuminating gas to be one of the commonest means of suicide in the cheap lodging houses of impoverished districts. Motor exhaust gas, which enjoyed a brief season of popularity, now accounts for rather few cases.

*Abortions.*—Figures on abortions are notoriously uncertain as to accuracy. In areas of strict registration most deaths from abortion are correctly reported, since reliable physicians are called before death or otherwise because of the danger of making an inaccurate report. Still the desire for secrecy is strong in many patients, and attempts to cover such deaths are not uncommon, even when the probability of criminal interference is not strong. It is obviously impossible to correlate deaths from abortions with the total number of abortions, because the latter must always remain an unknown quantity. The total number of deaths from abortions numbered 232, of which 40 were classed as due to criminal abortions. The low figure given for the latter class indicates the difficulty of proving criminal interruption of pregnancy, and the term "suspected criminal abortion" or "probable criminal abortion" cannot be used officially.

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9. Norris, Charles: Statistical Report of the Chief Medical Examiner of the City of New York, New York.

*Alcohol.*—Alcohol was listed as a primary cause of death in 303 cases, other than those due to delirium tremens, and as a directly associated factor in 336 cases. These 639 cases represent very largely acute intoxications, with evidence of alcoholism outstanding. Some of the cases in the second group occurred in persons who were addicted to the use of alcoholic stimulants and who acquired pneumonia or cardiac decompensation or renal disease following alcoholic sprees. Numerous accidents entailing death were associated with acute alcoholism. No coroner's physician would subscribe to the old saying that God has the drunkard especially under his watchful care.

Alcohol as a remote or associated cause of death cannot at present be properly evaluated. It is commonly stated that those who are chronically subject to alcohol have a marked liability to acute infections of the lungs and to shock from external trauma, including surgical operations. Yet such association is usually not mentioned in death certificates. This discrepancy between general statement and specific citation renders it easy for workers on either side of the question of the deleterious effects of alcohol to get medical support for their crusades.

In the work of coroner's physicians the number of cases included as cases of alcoholism must vary considerably if this diagnosis is accepted from history and anatomic findings alone, or if it must be supported by chemical examination of specimens removed at autopsy. In this respect it is of interest that chemically proved cases of alcoholism in the four years numbered 278.

Only 3 cases of pellagra were reported, and but 1 of these was definitely diagnosed as postalcoholic. Skin lesions are common among the human wrecks who are listed for coroner's investigation. Various forms of dermatitis, fungous infections and insect bites often combine to give mixed pictures which may confuse specific diagnosis. Often skin lesions are accepted as such a usual finding in cases from lower social strata that no attempt is made to find in them evidences of the cause of death. It seems probable that postalcoholic pellagra is similar to the usual form,<sup>10</sup> and since statistics indicate its increasing incidence, persons who are known to have been or may have been chronically alcoholic should be carefully scrutinized from the standpoint of pellagra.

*Homicides.*—Homicide was accountable for 1,984 deaths in this period. Much has been written about killing in the United States as one of its major evidences of crime. The homicide rate has been given as the highest in the world. Much of this killing goes unpunished. Indeed, capital punishment was evoked most sparingly in this period of four years. The disturbing feature is that a high percentage of

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10. Boggs, T. R., and Padget, P.: Bull. Johns Hopkins Hosp. 50:21, 1932.

such crimes are directed or inspired by organized outlaws of society who are able to scoff at threatened reprisals.

The coroner's physician is able in cases of criminal homicide to furnish much valuable evidence to the state. An autopsy certifies the exact cause of death and gives irrefutable proof of the means used in producing death, such, for example, as bullets recovered, specimens removed for chemical examination in poisoning, in asphyxiation or in drowning, and proof of the means of strangulation, stabbing or striking with heavy objects.

The killed persons numbered 1,699 males and only 282 females, which is in line with the sex distribution usually reported.

The colored race had 679 homicides with a rate of 68 per hundred thousand, as compared with 1,284 among the whites and a rate of 8.6. This is the reverse of the racial tendency toward suicides. The homicidal mania of the colored race is responsible for very high rates of homicide in certain cities of the South which have a high percentage of colored population.

Shooting accounted for 2,435 cases. These were divided into 1,424 homicides, 802 suicides, which have already been discussed, 158 accidents and 53 undetermined.

The fact that shooting disposed of 72 per cent of the victims in all homicides is ready evidence of a widespread ownership of firearms, as well as of deplorable sociopolitical developments, which encourage criminal use of these weapons.

Of other forms of homicide listed, cutting or stabbing accounted for 301 cases and blows on the head or body for 129. Scattered cases, only, were listed under various other methods of killing.

*Traumatic Septicemia.*—Coroner's cases in which septicemia caused death numbered only 103, or 26 per year. When it is remembered that this includes all cases arising from or associated with reported external trauma of whatever cause, some appreciation can be had of the remarkable advance in present day treatment of external trauma.

*Tetanus.*—Tetanus was recorded in 86 cases, or 21.5 per year. Though the number is low, these cases must be considered as sacrifices to public ignorance. By proper educational propaganda tetanus after Fourth of July celebrations has been made a rarity. The value of prophylactic treatment against tetanus after certain types of injuries generally could just as easily be made a matter of common knowledge.

*Excessive Heat.*—Heat was the cause of death in 176 coroner's cases. An attempt was made to subdivide the cases into those due to heat stroke and those due to heat exhaustion<sup>11</sup> but it was found impossible

11. Shattuck, G. C., and Hilferty, Margaret M.: Am. J. Trop. Med. 12:223, 1932.

to do this from the data and diagnoses given. So the inclusive term "excessive heat" was used as recommended for the official list of causes of death. The total number of deaths certified as due to heat numbered 596, so that the coroner's cases made up 29.5 per cent of the total. The general tendency during extremes of weather is to assign the extremes of temperature as causes of many deaths which should properly be classed otherwise. It is felt that the coroner's reports represent well selected cases from the standpoint of diagnosis.

The larger number of cases occurred in persons past the age of 50. This, too, in spite of the fact that industrial and other exposure to heat must have been much greater in the more active younger persons. Practically no deaths from heat occurred in children. Persons at the extremes of life have an imperfect heat-regulating mechanism, but infants are rarely killed by heat because they are shielded from it. If the aged could be shielded just as carefully from overactivity or over-exposure during hot weather there would undoubtedly be fewer tragedies due to heat among them.

*Epilepsy.*—Epilepsy, either of essential or jacksonian type, is often confused in the final state with recent injury to the head, delirium tremens and toxic psychosis. Coroner's cases are often woefully lacking in history, and clinical observations may have been only terminal or entirely lacking, so that factual material for reconstruction of a case that has reached the stage of death may be scanty. The lay witness, and occasionally the medical witness, is inclined to term almost any sort of convulsion "epileptic" and any delirium of sudden onset in a man "delirium tremens." Again the autopsy becomes the only method of investigation that gives facts.

Epilepsy was the cause of death in 52 cases. Some of the epileptic persons died in status epilepticus. Others perished in accidents resulting from epileptic attacks, such as falls or motor accidents with the epileptic person at the wheel. In the present mechanical age it may be difficult for an epileptic person attempting to lead an ordinary existence to avoid mechanical responsibilities, but certainly such risks to himself and others should be minimized.

*Delirium Tremens.*—Victims of this disorder came to their deaths almost always with intercurrent disease. Frequently the delirium was precipitated by an acute disease. Often also an attack was precipitated in a person chronically alcoholic by external trauma. What part was played by the intercurrent sickness or injury in causing death may be difficult to decide, so that, unless overbalanced by a definite history of inebriety, traumatic or toxic psychosis must be kept to the front until fully ruled out by autopsy.

## DISEASES AS CAUSES OF DEATH .

Sickness alone, or the so-called natural cause, is operative in many deaths that call for viewing by the coroner's physician. Especially is this true in sudden, perhaps unexplained deaths and deaths in which sickness is intercurrent with an external injury. In regard to the former group the coroner's physician is held responsible for certifying the cause of death as a matter of public policy, so that any possibly suspicious death may be properly investigated. In regard to the latter group many medicolegal questions arise as to criminal or civil responsibility.

In either group the autopsy is of prime importance. In sudden deaths arising largely on a basis of cardiovascular disease much pathologic investigation is still needed for better understanding of the processes involved. Most such pathologic material is lost to human benefit by being signed out on simple inquiry. When sickness complicates the course of trauma from external cause proper evaluation of the factors resulting in death can be made only by autopsy. All else is only approximation.

The total number of deaths from sickness was 5,618, or roughly one fourth of all the coroner's cases. In 1,739 of these, or a percentage of 30.9, autopsy was made.

*Cardiovascular Disease.*—Deaths from cardiovascular causes—heart disease including disease of the coronary artery, spontaneous hemorrhage of the brain and aneurysm—all combined numbered 3,586, or 63.8 per cent of all deaths from sickness.

The largest quota in this group was made up of the deaths from primary heart disease, which includes all forms leading to acute cardiac failure generally. There were 2,935 of these cases. There was also a complementary group of 240 cases in which death was due to disease of the coronary arteries.

A large proportion of the cases signed out as due to acute cardiac failure were "view" cases only. The rather typical history is that of a man of over 50 years with no history of current disease who collapsed while at some form of activity and died suddenly. Acute cardiac failure was given as the cause of death on the basis of probability, with the underlying pathologic condition not further proved.

Undoubtedly a much larger figure should be given for deaths from disease of the coronary arteries. The circumstances which render death reportable to the coroner quite naturally obtain in a considerable number of such cases. So the figure of 240 such deaths out of 3,175 deaths from acute cardiac failure of all forms indicates a probable insufficiency of examination into final causes.

Varied pathologic pictures were presented by the few autopsy reports in this group of sudden deaths. Besides cases of frank coronary throm-



bosis, there were many cases of chronic myocardial change mostly presumably on a vascular basis, unless associated with changes of the endocardium or pericardium suggesting other etiology. Ruptures of the wall of the heart were usually with recent coronary myomalacia, although rupture through a massive old scar of the left ventricle was seen. Massive hemato-pericardium appears to have resulted more often from rupture at the beginning of the aorta than from any other cause, this not necessarily as a consequence of syphilitic aortitis but sometimes as a result of formation of calcium plaques in the intima with development of dissecting aneurysm. A few cases of unsuspected acute endocarditis with fatal embolism and at least 1 case of acute annular ulcerative aortitis were found. There were several cases of ruptured unsuspected aneurysms in various locations. Massive cerebral hemorrhage accounted for several deaths, and likewise embolism after minor trauma, affecting the lungs or rarely the brain or the heart through a patent foramen ovale.

Spontaneous intracranial hemorrhage was diagnosed in 362 cases. Such a diagnosis in a case of immediate death without subsequent autopsy is much less in favor than formerly, since it is realized that intracranial hemorrhage does not as a rule kill instantly.

*Nephritis.*—Nephritis was assigned as the cause of death in 296 cases. The inclusion of these cases as coroner's cases was largely because of the tendency for coma to develop in persons with chronic nephritis on external trauma, exposure or toxemia. Acute glomerulonephritis, especially that superimposed on chronic nephritis, likewise developed with similar causes. Add to such etiology the fact that a considerable number of homeless persons have this disease and are without previous medical care, and that when they are found in late coma there is not sufficient time before death to rule out other possible causes besides nephritis. An autopsy certainly is demanded in all coroner's cases in which a person dies in coma and the clinical studies are not conclusive.

*Pneumonia.*—Pneumonia was written into the records of 1,608 cases, a number running surprisingly close to half that of the entire cardiovascular group.

There were 693 cases of primary pneumonia. The number of these was swelled by cases occurring in infants, many of whom died after a few days of what was considered a "cold," without medical attendance. Some aged persons also showed little reaction to such a serious infection and died suddenly at home with pneumonia unsuspected until autopsy. Persons with chronic alcoholism, especially those of lower economic strata, who slept off a bout with liquor exposed to inclement elements swelled the list of those who succumbed to pneumonia. Often derelicts, with no living place, they were sometimes found dead in out-of-the-way places, with pneumonia as the immediate cause of death.

Much harder to explain were the occasional cases of lobar pneumonia in adults with death following shortly after a dramatic onset of symptoms. An illustrative case may be cited. An apparently vigorous white woman of 40 years with no known complaint went into the city to transact business. While walking about she suddenly fell unconscious, was taken to a hospital and died four hours later without regaining consciousness. Complete autopsy disclosed only lobar pneumonia with an early gray stage of consolidation of the right lower and middle lobes. Such cases stress the possibility of pneumonia without the usual marked symptoms, which may go undiagnosed to either recovery or death.

A second group of cases of pneumonia is made up of those in which the condition was associated with external trauma, numbering 915. Here again persons at the extremes of life and persons who were debilitated comprise by far the largest portion. This was so striking in going over the cases that on reading "Patient, 65 years +, fall, with injury of several days' duration, and death" a finding of pneumonia could be reasonably predicted.

However, a considerable number of these cases were found in young persons and adults of middle life who had suffered trauma of varying severity. I believe, though without final proof, that in these pneumonia may arise on etiologic grounds quite different from those on which pneumonia arises in the less sturdy group. In the aged and debilitated persons the blame is generally laid on an inefficient circulation with congestion of the lungs, which prepares the ground for infection. It seems possible that in younger persons pulmonary infections may arise in areas in which circulation has been impaired by small emboli. The lungs as an efficient strainer for the venous blood undoubtedly intercept many foreign masses in the blood stream without symptoms of damage of the lungs arising. A shower of such small emboli might seriously impair the circulation of areas of the lung, without completely shutting it off, in a person with an efficient general circulatory function.

Still other factors were brought into play in late cases of exposure to gas, either illuminating or automobile exhaust gas, and in late cases of poisoning by depressant drugs such as opium, chloral, acetanilid, bromides and others. Many of the victims seemed to recover entirely from the original injuries, but within a few days contracted pneumonia, generally of lobular type, from which they died. In these cases depression of the respiratory function must be given a prominent rôle among etiologic factors, although some direct injury to the lungs is not ruled out.

Unfortunately, as to the whole question of pneumonia in cases of injury, this survey can cover only the fatal cases of injury, among

16,588 of which there were 915 which terminated in pneumonia. However, enough is shown of the seriousness of pulmonary complications to emphasize the need of clinical measures of prophylaxis. It is not too much to expect that the lag in the practical application of newer discoveries might be overcome to the extent of using as a routine the principle of hyperventilation of the lungs<sup>12</sup> in at least those cases of trauma severe enough to induce inactivity. Hyperventilation might be made a still more valuable procedure by using it periodically for some time after the injury.

*Status Thymicolymphaticus*.—This euphonious term was used rather sparingly in certificates of death. Only 41 cases were ascribed to this cause. The present reaction against such a diagnosis is well presented in the report of the British Commission.<sup>13</sup> But as long as the diagnosis is accepted the term will be found decorating death certificates. Its chief usefulness in the coroner's cases of sudden death and in those of obscure cause coming under the view of any lazy-minded practitioner of medicine arises from its eminent respectability and all-inclusive scope.

*Tuberculosis*.—Tuberculosis appears to be generally outside the scope of the coroner's inquiry, yet 233 cases were listed in which it was given as the primary cause of death. Certain points are brought out by these cases. First, that among the admittedly large number of people with active tuberculosis mingling in the general activities of life, accidents may occur. The shock of external trauma and the stress of the subsequent confinement show a likeliness to hasten the progress of existing active tuberculous lesions or indeed to light up new ones from latent foci. Next, an appreciable number of tuberculous people, perhaps euphoric, perhaps really carrying on fairly well, go to the end of their lives without medical care. The frequency of uncared-for cases among drug-users and prostitutes is too well known to need special comment here.

*Diabetes*.—The 54 cases in which this condition was found to be the cause of death came into the coroner's group principally for two reasons. One is that persons suffering from diabetes readily contract serious infections, perhaps ending in septicemia, from external trauma. The other is that many persons in whom diabetes is previously unrecognized or untreated die in diabetic coma.

*Pancreatitis*.—Acute hemorrhagic pancreatitis occurred in 5 cases. The frequently dramatic onset and short course of this disease may lead the laity to suspect it of resulting from external trauma. Especially is this true if the victim has recently undergone some special physical

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12. Henderson, Y.; Haggard, W., and Coburn, R. C.: *J. A. M. A.* **74**:783, 1920.

13. Young, M., and Turnbull, H. M.: *J. Path. & Bact.* **34**:213, 1931.

stress or injury. While cases have been reported<sup>14</sup> in which such association occurred, certainly medicolegally opinion should be reserved pending the careful ruling out of other more frequent causes.

*Peptic Ulcer.*—In 28 cases peptic ulcer caused death, by hemorrhage or perforation. Death by hemorrhage from a previously "silent" ulcer was noted. Again the vexing question arose as to the possibility of external trauma causing such a complication of ulcer of the stomach. Uncertainty was often added to by the passage of a considerable period of time between the supposed injury and the evidence of the complication.

*Hepatic Disease.*—Thirty-five deaths investigated by the coroner's physicians were due to diseases of the liver. Acute yellow atrophy was accountable for about two thirds of the cases. However, in the same length of time there were 94 fatal cases of acute yellow atrophy in the county. An increase of this disease has been feared from the extensive use of arsenical and coal tar medicines. Such cases, if recognized and properly reported, come under the coroner's investigation. Since the coroner's cases have been few as compared with the total number it appears that the feared increase has not occurred or that the reporting has been inaccurate.

*Acute Infections.*—Cases of acute infectious disease turned over to the coroner's service for investigation numbered 27. While such cases generally seem more properly to belong to the department of health, sudden death in a case in which the condition was undiagnosed, such as an asphyxial death with diphtheria, might call for an exercise of the coroner's power of complete investigation.

*Syphilis.*—This disease as a cause of death was recorded in 18 cases. I do not believe that this figure represents the total number of cases in which syphilis is a factor in causing death. In view of the type of cases included among the coroner's cases syphilis as a cause of death should rank more prominently. As long, however, as syphilis is a cause of death that carries not only a social but often also a financial penalty in the possible loss of life insurance or industrial insurance, the urge to concealment will operate. Besides allowing reasonable doubts to aid in salving the pride or economics of the family of the deceased, an examiner hesitates to expose himself to a possible suit for damages by including *de novo* the term "syphilis" in a death certificate, unless the diagnosis can be defended beyond peradventure.

*Tumors.*—Malignant tumors caused death in 157 cases. Most of these cases were those of persons in whom malignant growths had been diagnosed before death or in whom malignant growths had been sus-

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14. Stuart, M. C.: Northwest Med. 20:58, 1921.

pected but who had gone without medical attendance. Some exceptions were cases of persons who died in coma with primary or metastatic tumor of the brain, sometimes with superimposed acute hemorrhage, and of persons with minor injuries who had pathologic bone fractures with tumor, or of those whose tumors arose following external trauma.

As regards the first group of persons many were members of so-called religious cults which find their most fallow and remunerative fields among the victims of chronic diseases but at the end require a special public service to sign the death certificates.

The question of a single external trauma as the direct cause of malignant tumor remains yet to be proved, although there have been numerous coincidental possibilities observed among sarcomas. Carcinoma has not indicated such immediate relationship, except rarely.<sup>15</sup> Some of the cases in this group are those in which an effort was made to link the death etiologically with a reported external injury.

*Hemorrhage.*—Massive hemorrhage not induced by external trauma was found in 101 cases, aside from intracranial hemorrhage. Since the hemorrhage in itself was not the primary disease these cases were considered with those of the disease causing the bleeding. Hemorrhages from aneurysms, peptic ulcers and tuberculous lungs made up the majority, with hemorrhages from ruptured varices, especially of the esophagus, shown in a few cases. No case was listed in which a hemorrhagic diathesis was the cause of death.

A type of hemorrhage totally unrelated to the types mentioned is the late secondary hemorrhage occurring after external trauma. Only 3 such cases were given, so the interest of this type lies largely in its rarity. Since this type occurs with wound infection, the low number seems again to emphasize the present day advance in caring for wounds.

*Pulmonary Embolism.*—Massive pulmonary embolism after injury other than surgical operation terminated life in 57 cases. Embolism occurred with all grades of severity of injury and apparently with all degrees of motility of the subject. It was found in severely injured, bedfast patients and in slightly injured persons at full activity. It is not felt that any conclusion is permitted from such data, however, since the total number of nonfatal injuries with subsequent activity or inactivity cannot be determined, even though other variables could be evaluated.

#### OTHER CAUSES OF DEATH

Numerous other medical causes of death are represented by a few cases each. They were generally called into question by some suspicious circumstance about the death itself or because of lack of medical atten-

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15. Wells, H. G., and Cannon, P. R.: Tr. Chicago Path. Soc. **13**:183, 1930.

dance. Because of the scattering of these causes no special mention will be made of them.

#### SUMMARY

A review is presented of the 22,206 deaths investigated by the coroner's staff of Cook County, Ill., from November, 1928, to November, 1932. This number is 13 per cent of the total number of deaths in the area. These deaths occurred in 16,426 males and 5,425 females. The colored race, making up about 6 per cent of the population, contributed 11 per cent of the deaths. With the deaths grouped as to age, the percentage death rate is found to have been highest toward the extremes of life. In actual numbers, more deaths were found in males from 40 to 50 years of age, while in females the curve rose to an almost level plateau extending from 20 to 70 years of age. There were 355 fetuses; the causes of death remained mostly unknown. Different seasons of the year were not found to influence appreciably the total number of cases.

There were 12,927 cases examined in undertakers' morgues with an autopsy percentage of 18.7, 5,240 at the Cook County morgue, in 53.4 per cent of which autopsies were made, and 3,684 at various hospitals, in 24.8 per cent of which autopsies were made. The percentage of autopsies in all cases in all places of examination was 27.6. Death certificates were signed by coroner's physicians in 5,628 cases, while in the remainder, 16,223 cases, the report of death was completed by inquest.

Deaths from external traumas numbered 16,588. The following types of cases of injury resulting in death are considered: accidents of transportation, 10,768; suicides, 2,723; homicides, 1,984; burns, 640; falls, 611; industrial accidents, 610; asphyxiations, 470; alcoholic injury as a primary factor, 303, and as an associated factor, 336; abortions, 232; excessive heat, 176.

The duration of life following injury was short in most cases, with 17,401 of the cases terminating in death in less than twenty-four hours. In the remaining cases, 4,450, not including those of fetuses, the histories varied in length up to one year or more.

Cases in which sickness was the only or the associated cause of death numbered 5,618. Specific diagnoses were given as follows: Among cardiovascular diseases, primary heart disease, 2,935, with a complementary group of coronary arterial disease, 240, and spontaneous intracranial hemorrhage, 362; pneumonia as the primary cause of death, 693, and associated with injury or other cause, 915; nephritis, 296; tuberculosis, 233; malignant tumor, 157; massive hemorrhage not associated with external trauma, 101; massive pulmonary embolism

after disease or injury other than surgical, 57; diabetes, 54; status thymicolymphaticus, 41. Numerous other diagnoses were given with few deaths.

This review is presented as an attempt to stress the general importance of the great mass of coroner's deaths both as to sociopolitical and economic significance and as to the valuable medical lessons to be derived from the course of injuries or diseases, either as separate entities or when intercurrent.

# General Review

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## ENDOMETRIAL HYPERPLASIA

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AND

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Endometrial hyperplasia is an important gynecological condition characterized by profuse and irregular hemorrhages, hyperplastic endometrium and absence of functioning corpora lutea. It is an important disorder of the menstrual function, and before considering it in detail it seems wise to review a few important facts concerning the normal physiology of menstruation. Recent work has advanced knowledge of this process considerably and given some insight into it.

### THE MENSTRUAL MECHANISM

The normal, healthy woman menstruates at more or less regular intervals of about twenty-eight days. Following menstruation the endometrium undergoes a gradual proliferation up to the time of ovulation, which occurs at about the ninth postmenstrual day. Then the epithelium ceases to proliferate and begins to undergo secretory changes. When menstruation begins, the endometrium rapidly disintegrates and is cast off. Following menstruation the epithelium grows out from the glands and covers the desquamated area, and the proliferation starts again. These cyclic changes in the endometrium are dependent on cyclic changes occurring in the ovary. In the latter organ there is a period of follicular ripening following menstruation. On or near the ninth postmenstrual day this process terminates in the rupture of the follicle and the discharge of the ovum. Following follicular rupture, or ovulation, the lining cells of the ruptured follicle begin to grow and form the corpus luteum. During the process of follicular ripening the follicles secrete a hormone which stimulates the growth of the endometrium. Principles having an estrogenic action or extracts containing such principles have been variously designated (female sex hormone, theelin, amniotin, prog-

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non, estrin, etc.). Since the hormone has the capacity to produce estrus in the spayed animal, the term estrogenic substance has seemed particularly appropriate. In the ovary that substance is found in the graafian follicle, interstitial tissue and corpus luteum. However, the follicles are generally considered as the main source of this secretion. Substances of a similar nature are found in the blood of the menstrual discharge, in the placenta, and in the blood and urine during pregnancy. A chemically pure substance having, so far as is known, the same physiologic effects has been isolated by Doisy, Veler and Thayer<sup>1</sup> and by Butenandt<sup>2</sup> from the urine of pregnant women. Following ovulation, the follicle undergoes a series of changes which result in the formation of the corpus luteum. Fraenkel<sup>3</sup> first definitely proved that this structure had the properties of an endocrine gland. Recently Corner and Allen<sup>4</sup> isolated a hormone from the corpus luteum. This hormone has no effect on the endometrium of the castrated animal unless the endometrium has previously been sensitized with the estrogenic hormone. If so, the proliferative changes produced in the endometrium as a result of that hormone are modified by the action of the corpus luteum hormone, and premenstrual or progestational changes occur. These two hormones provide the stimulus for the endometrial changes taking place in the interval between the end of one period and the beginning of the next. Those before ovulation are caused by the estrogenic substance and those following ovulation by the combined effects of the estrogenic and the corpus luteum hormone.

The premenstrual phase is terminated by the onset of menstruation. The actual cause of the menstrual bleeding is considered by Hartman, Firor and Geiling<sup>5</sup> to be a special menstrual hormone which originates in the anterior pituitary gland. On the other hand, Saiki<sup>6</sup> failed to confirm their findings and produced good evidence that menstruation is a degenerative process resulting from a decline in the ovarian hormones. According to this view, the endometrium is dependent on the hormones for its growth stimulus. When the growth stimulus is removed, growth stops and the tissue regresses to its former condition. In the human endometrium and in that of the monkey this regression is associated with certain vascular phenomena which result in menstruation. It has been known for some time that removal of the human ovaries in

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1. Doisy, E. A.; Veler, C. D., and Thayer, S.: *J. Biol. Chem.* **86**:499, 1930.

2. Butenandt, A.: *Deutsche med. Wchnschr.* **55**:2171, 1929.

3. Fraenkel, L.: *Arch. f. Gynäk.* **91**:705, 1910.

4. Corner, G. W., and Allen, W. M.: *Am. J. Physiol.* **88**:326, 1929. Allen, W. M.: *Am. J. Physiol.* **92**:174, 1930.

5. Hartman, C. G.; Firor, W. M., and Geiling, E. M. K.: *Am. J. Physiol.* **95**:662, 1930.

6. Saiki, S.: *Am. J. Physiol.* **100**:8, 1932.

the premenstrual phase results in the premature appearance of the menstrual flow. This clinical fact lends support to the views of Saiki, as does the earlier work of Allen <sup>7</sup> who noted menstruation in monkeys following injections of theelin. Recently Werner and Collier <sup>8</sup> observed menstruation in castrated women following injections of theelin. Kaufmann <sup>9</sup> and Clauberg <sup>10</sup> have gone a step further and noted menstruation in castrated women after injections of commercial preparations of the estrogenic and the corpus luteum hormone.

The important studies of Smith and Engle <sup>11</sup> and of Zondek and Aschheim <sup>12</sup> have conclusively demonstrated the dependence of the ovary on the anterior lobe of the hypophysis. If the ovary is removed the sex cycle stops, but if an excess of the hormone from the anterior lobe of the pituitary gland is added there is a marked ovarian reaction consisting of follicular ripening, ovulation and formation of corpus luteum. Zondek <sup>13</sup> expressed the belief that the anterior lobe has a sex factor stimulating follicular ripening and one stimulating luteinization. He designated these factors as prolan A and prolan B respectively. (In this country the term prolan is often used to designate the anterior pituitary-like hormone found in the urine of pregnant women.) Other investigators are inclined to believe that prolan A and prolan B represent quantitative variations of a single sex hormone. The process of ovulation or follicular rupture initiates the formation of the corpus luteum. Ovulation occurs spontaneously in most animals; in the cat, ferret and rabbit it occurs only after copulation. In the rabbit ovulation can be induced by injections of the urine of pregnant women and by injections of a substance obtained from the anterior lobe of the hypophysis. Normally, ovulation in the rabbit occurs from ten to twelve hours after copulation; it is thought that the act of copulation sets up a nervous impulse which stimulates the anterior pituitary lobe to secrete a hormone which produces ovulation. In human beings, ovulation occurs spontaneously. No data are available on the effect of nervous impulses on human ovulation. Under certain conditions these might become a factor. Allen <sup>14</sup> considered such a possibility likely. While the hypophysis has a profound action on the ovary, the ovary has a counter effect on the

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7. Allen, Edgar: *Contrib. Embryol.* **19**:1, 1927.

8. Werner, A. A., and Collier, W. D.: *J. A. M. A.* **100**:633, 1933.

9. Kaufmann, C.: *Klin. Wchnschr.* **12**:217, 1933.

10. Clauberg, C.: *Zentralbl. f. Gynäk.* **57**:1461, 1933.

11. Smith, P. E., and Engle, E. T.: *Am. J. Anat.* **40**:159, 1927.

12. Zondek, B., and Aschheim, A.: *Arch. f. Gynäk.* **130**:1, 1927.

13. Zondek, B.: *Die Hormone des Ovariums und das Hypophysenvorderlappens*, Berlin, Julius Springer, 1931; *Klin. Wchnschr.* **9**:245, 1930.

14. Allen, Edgar: *Sex and Internal Secretions*, Baltimore, Williams & Wilkins Company, 1932, p. 431.

hypophysis. Several investigators have mentioned this interaction, notably Leonard, Meyer and Hisaw<sup>15</sup> and Moore and Price,<sup>16</sup> and it need not be considered here in detail. It suffices to say that the hypophysis stimulates the ovary, and the estrogenic factor, one of the ovarian secretions, depresses the hypophysis. Alterations in this balance could easily lead to disturbances in the menstrual rhythm. However, the complete solution of the hypophyseal-ovarian relationship is not yet at hand. The importance of this relationship in the study of endometrial hyperplasia will be considered later.

The condition known as endometrial hyperplasia has now been separated from the disorders of menstruation and stands as a clinical and pathologic entity. According to Olshausen,<sup>17</sup> as early as 1846 the French knew of a polypoid condition of the endometrium associated with hemorrhages. Olshausen studied the condition and gave it the name of "endometritis fungosa." Seven years later, a German gynecologist, Brennecke,<sup>18</sup> published the contribution which opened the way to the present day advances. He was the first to describe the absence of corpora lutea in the ovaries, and suggested that an ovarian disorder was responsible for the endometrial changes. The full significance of Brennecke's observations was not appreciated, because at that time the endometrial cycle was still unknown, and many normal conditions were described as forms of endometritis. In 1900, Thomas Cullen<sup>19</sup> described the condition and recognized it as an abnormal hyperplastic process of benign character, separate and distinct from endometritis. He named it "endometrial hyperplasia." However, until after Hitschmann and Adler<sup>20</sup> had described the normal histology of the endometrium in 1908, it was impossible to differentiate completely the normal from the pathologic process. Their work laid the foundation for the further study of endometrial lesions. This was forthcoming in the work of Schroeder<sup>21</sup> and to him and his pupils is due most of the credit for the present

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15. Leonard, S. L.; Meyer, R. R., and Hisaw, F. L.: *Endocrinology* **15**:17, 1931.

16. Moore, C. R., and Price, D.: *Am. J. Anat.* **50**:13, 1932.

17. Olshausen, R.: *Arch. f. Gynäk.* **8**:97, 1875.

18. Brennecke: *Arch. f. Gynäk.* **20**:455, 1882.

19. Cullen, Thomas: *Cancer of the Uterus*, New York, D. Appleton and Company, 1900.

20. Hitschmann, F., and Adler, L.: *Monatschr. f. Geburtsh. u. Gynäk.* **27**:1, 1908.

21. Schroeder, R.: *Arch. f. Gynäk.* (a) **98**:81, 1912; (b) **104**:27, 1915; (c) *Zentralbl. f. Gynäk.* **44**:755, 1920; (d) in Halban, Joseph, and Seitz, Ludwig: *Biologie und Pathologie des Weibes*, Berlin, Urban & Schwarzenberg, 1924, vol. 3, p. 921; (e) Schroeder, R.; Kessler, R., and Tietz, K.: *Zentralbl. f. Gynäk.* **57**:11, 1933.

knowledge of endometrial hyperplasia. As a result of Schroeder's work, the Germans have recognized the condition and written voluminously about it. In England and America the condition has not commanded the attention to which it is entitled. In this country the fundamental contribution of Cullen<sup>19</sup> and the later work of Emil Novak<sup>22</sup> and Fluhmann<sup>23</sup> have done much to advance knowledge of the subject.

#### ETIOLOGY OF ENDOMETRIAL HYPERPLASIA

From a correlative study of ovarian and uterine changes Schroeder<sup>21b</sup> correctly inferred that the uterine changes before ovulation were caused by a hormone from the follicle, and those after ovulation by a hormone from the corpus luteum. In hyperplasia he found no corpora lutea and he concluded from this that the endometrial changes were the result of a failure of ovulation. This resulted in a persistent follicle, which stimulated the hyperplastic growth of the endometrium. The eventual cause was considered to be a disharmony of the glands of internal secretion. Meyer<sup>24</sup> placed a somewhat different interpretation on the ovarian findings. He considered that the stimulus arose from a succession of ripening follicles which never came to ovulation, rather than from one persistent follicle. He was unable to find any corpora lutea in his cases.

As a general rule the presence of corpora is the exception; they have been reported in only a small percentage of cases. Novak and Martzloff<sup>25</sup> observed corpora lutea in four cases of hyperplasia. Babes,<sup>26</sup> in a report from Meyer's laboratory, analyzed twenty-five cases and found corpora in three. Shaw<sup>27</sup> found two recently ruptured follicles and a recent corpus luteum. Fluhmann<sup>23b</sup> found no corpora in five instances. We believe that the occasional occurrence of corpora can probably be explained either by diagnostic errors, nonfunctioning corpora or spontaneous and induced ovulation in the course of the disease. We have recently observed three cases in which a definite hyperplasia was transformed into a normal premenstrual endometrium and normal

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22. Novak, Emil: (a) *J. A. M. A.* **63**:617, 1914; (b) *Am. J. Obst. & Gynec.* **75**:996, 1917; (c) *J. A. M. A.* **75**:292, 1920; (d) *M. J. & Rec.* **130**:481, 1929; (e) *Endocrinology* **15**:273, 1931; (f) *South. M. J.* **25**:261, 1932; (g) in Curtis, A. H.: *Obstetrics and Gynecology*, Philadelphia, W. B. Saunders Company, 1933, vol. 3, p. 124.

23. Fluhmann, C. F.: (a) *J. A. M. A.* **93**:1136, 1929; (b) *Surg., Gynec. & Obst.* **52**:1051, 1931; (c) *Endocrinology* **15**:177, 1931; (d) *Am. J. Obst. & Gynec.* **21**:455, 1931; (e) *Proc. Soc. Exper. Biol. & Med.* **31**:54, 1933.

24. Meyer, R.: *Arch. f. Gynäk.* **113**:259, 1920.

25. Novak, E., and Martzloff, K. H.: *Am. J. Obst. & Gynec.* **8**:385, 1924.

26. Babes, A. A.: *Arch. f. Gynäk.* **122**:448, 1924.

27. Shaw, Wilfred: *J. Obst. & Gynaec. Brit. Emp.* **36**:1, 1929.

menstruation ensued. In each case the cervix had been stimulated by the passage of a biopsy instrument, and it is possible that the nervous stimulation from this procedure played a part in bringing about ovulation. In order for a hyperplasia to be converted into a premenstrual endometrium, ovulation and the formation of corpus luteum must have taken place. If the operation had been performed on the first or second day after ovulation, we might have obtained the picture of a hyperplastic epithelium with early corpora lutea in the ovaries. In the cases referred to had the specimens been obtained later, the epithelium might have been perfectly normal in appearance, and the expected menstruation might have been normal, as it was in our cases. These facts indicate that the hyperplastic picture may change and that ovulation and the formation of corpus luteum may terminate the disease. In such an instance there is a transitory phase in the conversion of the hyperplastic endometrium into the premenstrual endometrium. Early in this transitory phase the endometrium may be hyperplastic, and yet the condition known as endometrial hyperplasia cannot be said to exist. Spontaneous remissions have been known to all authors, and since a remission can occur only by ovulation and the formation of corpus luteum, it is not surprising that early corpora lutea have been reported in association with a hyperplastic endometrium. When mature corpora are found in the disease they are probably nonfunctioning, or there is an error in the interpretation of the endometrium. Increasing experience has confirmed our belief that endometrial hyperplasia and functioning ripe corpora lutea do not occur simultaneously.

Novak <sup>22e</sup> in a recent contribution stressed the importance of persistent and excessive follicular stimulation as the cause of the characteristic hyperplasia. Experimental proof of Schroeder's and Meyer's contention of an abnormal follicular stimulation producing the hyperplastic changes was put forth by Burch, Williams and Cunningham.<sup>28</sup> By injections of the estrogenic hormone these authors were able to produce in the endometrium of spayed rodents changes in growth which were quite similar to the changes observed in human hyperplasia. Injections of the corpus luteum hormone failed to produce such changes. Moreover, these changes were reproduced in two instances by injection of material from the cystic follicles in cases of hyperplasia. Wolfe, Campbell and Burch <sup>29</sup> were later able to reproduce successfully all the characteristic features of the disease in rats and guinea-pigs by injecting, over a longer period of time, a purified, crystalline estrogenic hormone,

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28. Burch, J. C.; Williams, W. L., and Cunningham, R. S.: *Surg., Gynec. & Obst.* **53**:338, 1931.

29. Wolfe, J. M.; Campbell, M., and Burch, J. C.: *Proc. Soc. Exper. Biol. & Med.* **29**:1263, 1932.

theelin. On the other hand Spivack<sup>30</sup> was unable to produce experimental hyperplasia by injecting 10 Allen-Doisy units of a commercial estrogenic hormone every other day until fifteen doses were given. Hofbauer<sup>31</sup> was the first to produce the condition experimentally. He injected into guinea-pigs a substance freshly obtained from the anterior hypophyseal lobe. He considered the condition as a manifestation of an overactive anterior lobe. Oskar Frankl<sup>32</sup> conceded the correctness of Hofbauer's contention of overactivity of the anterior lobe for a limited number of cases but stated that local inflammatory conditions in the ovary are a far more frequent cause of hyperplasia than overactivity of the anterior lobe. Burch, Wolfe and Cunningham<sup>33</sup> were unable to produce the condition experimentally by utilizing Lipschütz' <sup>34</sup> method of partial castration, and they stressed partial castration as an important predisposing cause in human beings. It is interesting to note that of eighteen patients with endometrial hyperplasia reported on by Graves,<sup>35</sup> four, or 22 per cent, had had a previous unilateral oophorectomy, and that of eight patients with menorrhagia and metrorrhagia reported on by Esch,<sup>36</sup> three had had a previous unilateral oophorectomy.

Shaw<sup>27</sup> stated the belief that the primary change is in the endometrium itself, and stated:

Thus far the explanation I have to offer to account for the features of this disease may be summarized as follows: the polypoidal endometrium reacts upon the ovaries to produce an inhibition of follicle ripening, or of ovulation or of full formation of the corpus luteum; in turn, the ovaries respond by producing continuously a toxin, identical in type with that causing the disintegration of the premenstrual endometrium in normal menstruation. In consequence, disintegration of the superficial layers of the polypoidal endometrium occurs.

Likewise, Babes<sup>26</sup> suggested that, in some cases, the picture may be due to a peculiarity of the epithelium itself. No experimental proof, however, has been brought forward to support these assumptions. The latest chapter has been added by Kaufmann,<sup>9</sup> who produced the condition in castrated women by injections of a benzoic acid ester of the dihydro-estrous hormone, and one is compelled to conclude that the endometrial changes are the result of the action of the estrogenic hormone.

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30. Spivack, Mary: *Surg., Gynec. & Obst.* **54**:733, 1932.

31. Hofbauer, J.: *Zentralbl. f. Gynäk.* **54**:2569, 1930; *Surg., Gynec. & Obst.* **52**:222, 1931.

32. Frankl, O.: *Zentralbl. f. Gynäk.* **55**:68, 1931.

33. Burch, J. C.; Wolfe, J. M., and Cunningham, R. S.: *Endocrinology* **16**:541, 1932.

34. Lipschütz, A.: *The Internal Secretions of the Sex Glands*, Baltimore, Williams & Wilkins Company, 1924.

35. Graves, W. P.: *Am. J. Obst. & Gynec.* **20**:500, 1930.

36. Esch, P.: *Zentralbl. f. Gynäk.* **54**:19, 1930.

Burch, Williams, Wolfe and Cunningham<sup>37</sup> pointed to a disturbance of the hypophyseal-ovarian relationship as an explanation for the abnormal production of the estrogenic substance, while Novak<sup>22f</sup> suggested that there is a disordered balance between the motivating hormones of the anterior lobe.

The ovarian changes have been carefully studied and, in addition to the absence of corpora lutea, the presence of follicular cysts is a prominent feature. Babes<sup>26</sup> estimated that there are one or two large follicular cysts in at least 60 per cent of the cases. Shaw<sup>27</sup> considered them an almost invariable accompaniment. The opposite ovary is shrunken and sclerotic. The cysts may be lined with a layer of granulosa cells, but frequently the latter have disintegrated. Babes<sup>26</sup> mentioned the scarcity of primordial follicles and the presence of degenerated ova in those present. He believed that his observations supported Meyer's theory of continuously ripening follicles rather than Schroeder's view of a persistent follicle. The cysts may attain the size of a small grapefruit and are frequently large enough to be palpated on bimanual examination. They may persist in an ovary almost indefinitely or may disappear in a few weeks. Patients have been observed in whom cysts were first noticed in one ovary, from which they disappeared only to reappear in the other ovary. The cysts are generally regarded as a manifestation of an endocrine disorder affecting the ovulatory mechanism, although some believe that thickening of the tunica albuginea can prevent ovulation in a purely mechanical way. The occurrence of the disease as a remote sequel to pelvic inflammatory conditions lends support to this view, but as yet there is no statistical or experimental evidence to confirm the theory.

The ovarian findings have not cast any light on the question of hypofunction or hyperfunction of the ovary. Most authors consider that there is a hyperfolliculism, and this implies that there is always a true increase in the amount of estrogenic substance available to act on the endometrium. It is conceivable that when there is an especially marked tendency to the production of estrogenic substance, the hypophysis is so depressed by this excess that it never reaches the peak necessary for ovulation. In the younger group of patients this may be likely. In other patients the ovary may be particularly hard to stimulate, and the production of estrogenic substance may be at a lower level than normal. In this group the hypophysis may be working at a level higher than normal, although not high enough to produce ovulation in the refractory ovaries. This seems likely in some of the older patients. No histologic criteria indicating the degree of secretion of the estrogenic hormone

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37. Burch, J. C.; Williams, W. L.; Wolfe, J. M., and Cunningham, R. S.: *J. A. M. A.* **97**:1859, 1931.

have been developed, and the question of hypersecretion and hyposecretion of that hormone must be settled by the results obtained from quantitative studies of the blood throughout the course of the disease.

No data on the histologic changes in the pituitary gland in human hyperplasia have been encountered. Wolfe, Phelps and Cleveland<sup>38</sup> studied this question in rats and guinea-pigs subjected to extreme partial castration. Some of the animals failed to come into estrus after the operation; in such animals the pituitary gland showed an increase in the number and size of the basophilic cells, which became modified into castration cells. In other animals continuous estrus, associated with hyperplasia of the endometrium, was observed. The anterior pituitary lobes of such animals exhibited a decrease in the relative percentage of basophils; the basophils present contained little granular material. The full significance of these findings is not apparent, but they show clearly that different ovarian conditions are reflected in the histologic structure of the anterior pituitary lobe.

The relation of the thyroid gland to hyperplasia has often been suggested, largely on account of the successful use of thyroid extract in an occasional case. Opinion, however, is divided on this point, largely as a result of insufficient data. Owing to the relative rarity of the juvenile types, in which the point is of the utmost importance, and to the great frequency of the menopausal types, in which glandular therapy is rarely considered, large series of basal metabolic rates are not available. Gyllensvärd<sup>39</sup> noted the question of whether a disturbance of thyroid function may not be a cause of the disease in the adolescent groups. He reviewed the previous work of other authors and reached the conclusion that, even though it must be regarded as probable that in a few cases there is a disturbance of thyroid function, such a disturbance cannot, at least with the present methods of investigation, be demonstrated in a sufficiently large number of cases to be justifiably regarded as the cause of the hemorrhages. The evidence regarding the part played by the thyroid gland in menstrual disorders has been well summarized by Mazer and Goldstein.<sup>40</sup> They found the basal metabolic ratio below minus 15 in only four of one hundred and three cases. Sehrt,<sup>41</sup> McCarrison<sup>41</sup> and Bram<sup>41</sup> expressed the belief that menorrhagia is a symptom of hypothyroidism. Warfield,<sup>41</sup> however, stressed the infrequency of men-

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38. Wolfe, J. M.; Phelps, D., and Cleveland, R.: *Anat. Rec.* **55**:85 (supp., March 25) 1933.

39. Gyllensvärd, N.: *Acta obst. et gynec. Scandinav.* **11**:423, 1931.

40. Mazer, C., and Goldstein, L.: *Clinical Endocrinology of the Female*, Philadelphia, W. B. Saunders Company, 1932.

41. Quoted by Mazer and Goldstein.<sup>40</sup>



strual disorders in hypothyroidism. The ultimate solution of this question awaits further clinical research on proved cases of endometrial hyperplasia.

From time to time there have been reported cases in which menstrual disorders have been attributed to nervous causes. Novak<sup>22a</sup> reported two cases and Miller<sup>42</sup> presented an exceedingly interesting series of cases illustrating this point. At the present time we have a similar case under observation. The relationship of ovulation to nervous stimulation has already been discussed, and the possibility of a nervous factor in the production of the disease is admitted, but so far proof of its occurrence is lacking.

Since hyperplasia is closely connected with disorders of the hypophyseal-ovarian relationship, it is not unreasonable to expect tumors of the hypophysis and proliferating tumors of the ovary to be associated with the disease.

According to Henderson,<sup>43</sup> adenomas of the pituitary gland produce sexual dysfunction as a result of compression of the normal portion of the gland. The two common types of adenomas, acidophilic and chromophobic, do not differ in the type of sexual dysfunction produced. The sex hormone is considered as being derived from the basophilic cells. Basophilic adenomas are less common than acidophilic and chromophobic, and no reference to them in connection with endometrial hyperplasia has been noted.

While tumors of the pituitary gland are not related to the disease, the relationship of proliferative tumors of the ovary to endometrial hyperplasia is well established. On several occasions Schiffmann<sup>44</sup> called attention to these tumors. He considered the endometrial changes to be the result of a hormone produced in the tumor, while Oscar Frankl,<sup>45</sup> in reporting a fibroma associated with endometrial hyperplasia, stressed the possibility of the mechanical disturbance of circulation creating a stimulus for endometrial growth. Some of the earlier authors mentioned that congestion may provide a stimulus for endometrial growth. While this general assumption has not been disproved, most authors consider that the stimulus from proliferative tumors is due to a hormone and not to passive hyperemia resulting from the tumor. Schuschania's<sup>46</sup> observation of the estrogenic hormone in the blood and urine of one patient supports this contention. As regards type, granulosa cell tumors, Brenner's tumors, fibroma, carcinoma and sarcoma have all been mentioned. Mistakes in classification have been frequent, and the present

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42. Miller, J. A.: *M. J. & Rec.* **134**:84, 1931.

43. Henderson, W. R.: *Endocrinology* **15**:111, 1931.

44. Schiffmann, Josef: *Arch. f. Gynäk.* **150**:159, 1932.

45. Frankl, O.: *Zentralbl. f. Gynäk.* **53**:9, 1929.

46. Schuschania, P.: *Zentralbl. f. Gynäk.* **54**:1924, 1930.

tendency is to classify most of these tumors in the group of granulosa cell tumors. The appearance of the tumor after the menopause produces somewhat of a sexual regeneration. The endometrium is hyperplastic, and irregular bleeding occurs. Ovarian tumors associated with post-menopausal hyperplasia are always suggestive of malignancy. Schiffmann<sup>44</sup> and TeLinde<sup>47</sup> stressed the practical importance of this association. The latter's excellent contribution has done much to arouse interest in this subject.

Studies on the hormone content of the blood and urine of patients with endometrial hyperplasia are suggestive, but they are of significance only when they include a comparison with the normal. Since the almost simultaneous discovery by Frank<sup>48</sup> and by Lowe<sup>49</sup> of estrogenic substance in the circulating blood of normal women, fairly definite normal values have been established. Considerable work on this subject has been done by Frank and his collaborators.<sup>50</sup> These investigators worked out a more or less standardized test for the determination of the presence of the estrogenic factor in the blood which is of importance in the diagnosis of certain gynecological conditions. Briefly, their findings in a large series of normal women were as follows: Demonstrable amounts of estrogenic substance were found in the circulating blood in the premenstrual phase of the menstrual cycle. In a few instances the substance was found as early as ten days before the onset of the next menstrual period; <sup>50c</sup> in practically all the women it was found in the blood three days before the expected menstruation. At the onset of menstruation the estrogenic substance disappeared rapidly from the circulating blood, but was found highly concentrated in the first blood of the menstrual discharge.<sup>50c</sup> These pioneer studies of Frank and his co-workers have been subsequently confirmed by others, notably by Mazer and Hoffman,<sup>51</sup> Mazer and Goldstein,<sup>40</sup> Hirsch<sup>52</sup> and Siebke.<sup>53</sup>

In a recent and important contribution Fluhmann<sup>23e</sup> described the utilization of the methods of Robson and Wiesner<sup>54</sup> and of Meyer and Allen<sup>55</sup> (who found that a subcornifying dose of estrogenic hormone resulted in mucification of the vaginal mucosa of spayed mice) as a

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47. TeLinde, R. W.: *Am. J. Obst. & Gynec.* **20**:552, 1930.

48. Frank, R. T.; Frank, M. L.; Gustavson, R. G., and Weyerts, W. W.: *J. A. M. A.* **85**:510, 1925.

49. Loewe, S.: *Klin. Wchnschr.* **4**:1407, 1925.

50. Frank, R. T.: (a) *The Female Sex Hormone*, Springfield, Ill., Charles C. Thomas, Publisher, 1929; (b) *J. A. M. A.* **97**:1852, 1931; (c) **90**:106, 1928.

51. Mazer, C., and Hoffman, J.: *Am. J. Obst. & Gynec.* **17**:186, 1929.

52. Hirsch, H.: *Arch. f. Gynäk.* **133**:173, 1928.

53. Siebke, Harold: *Zentralbl. f. Gynäk.* **53**:2450, 1929.

54. Robson, J. M., and Wiesner, B. P.: *Quart. J. Exper. Physiol.* **21**:217, 1931.

55. Meyer, R. K., and Allen, W. M.: *Science* **75**:111, 1932.

test for that hormone in the blood of normally menstruating women. In his experiments he injected fresh blood serum into spayed mice, which were killed on the third or fourth day after the first injection. The vaginae were fixed and sectioned. In mice which had received serum containing a detectable amount of estrogenic substance the vaginal epithelium was mucified. In his total series he tested the blood of fifty-seven women during the different phases of the menstrual cycle. The blood of ten women in the early proliferative stage, from the fourth to the seventh day of a thirty day cycle, was examined; two tests were positive and eight negative. Nine of fifteen tests made in the late stage of proliferation were positive; fifteen of eighteen tests made in the early phase of secretion were positive, and six of seven tests made in the last stages of secretion were positive. The blood of three women within forty-eight hours of menstruation was examined; only one test was positive. These results seem to be of special importance; they compare well with the findings of Frank and his co-workers; also they offer a more exact method for the determination of small amounts of estrogenic substance than does cornification of the vagina. It is to be hoped that this excellent method of study will be extended to abnormal conditions.

The estrogenic substance has also been detected in the urine of normally menstruating women. Frank <sup>50b</sup> found that normal women may secrete from 800 to 1,500 mouse units of that substance during one menstrual cycle; two periods of maximum excretion were found, one near the time of ovulation and the other one or two days before menstruation. Kurzrok and his collaborators <sup>56</sup> reported that normal women secrete from 10 to 20 units of estrogenic substance per liter of urine. They found little variation in the amount secreted during the various phases of the menstrual cycle.

Since endometrial hyperplasia appears as the result of an abnormal stimulus in the secretion of estrogenic substance several investigators have tested the blood and urine of patients with this condition for the presence of the hormone, but the results have been somewhat variable. Frank <sup>50b</sup> studied several groups. In a series of over a dozen adolescents with endometrial hyperplasia he found that both the urine and the blood contained an excess of estrogenic hormone. He also reported that the blood of women showing climacteric bleeding of a functional type exhibited a high level of that hormone; in some instances some cyclic variation in the amounts present was noted. The endometrial changes were closely similar to those in the pubertal group. In contrast, he failed to find such high levels of estrogenic substance in the blood of adult patients exhibiting functional menorrhagia and metrorrhagia. Occasionally the

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56. Kurzrok, R.; Boylan, James; Goldman, S., and Creelman, M.: *Endocrinology* 16:361, 1932.

blood of these patients contained small amounts of the hormone, which, in such instances, exhibited cyclic variations. The endometrium of the aforementioned adult patients was either of the resting or of the stationary hyperplastic type. Mazer and Goldstein,<sup>49</sup> in agreement with Frank, failed to find the estrogenic substance in the blood of mature women with dysfunctional uterine bleeding. Furthermore, in contrast to Frank, they failed to find the substance in the blood in a high percentage of cases of adolescent girls suffering from the bleeding of puberty. They demonstrated its presence in the blood in only four of fifteen patients with functional preclimacteric bleeding. (These investigators reported no observation on the endometrium.) Siebke,<sup>53</sup> utilizing the method of Frank and Goldberger, studied the blood of sixteen patients with glandular hyperplasia of the endometrium. In ten instances the blood contained the estrogenic principle; in these cases the patients were not bleeding or had been bleeding for only a relatively short period. In nine observations made on the blood of patients who had been bleeding for a longer period (from two and a half weeks to three months) no estrogenic substance was found in the blood. Zondek<sup>57</sup> found the substance in the urine of patients during the amenorrheic periods of "hyperhormonal amenorrhea." The ovaries of these patients showed follicles which exceeded normal follicles in size and hormone content. In patients exhibiting polymenorrhea, usually associated with cystic hyperplasia of the endometrium, Kurzrok and his associates<sup>58</sup> found that as much as 60 units of estrogenic substance per liter of urine sometimes occurred. On the other hand, Mazer and Hoffman<sup>51</sup> failed to find the hormone in the urine of six patients exhibiting endometrial hyperplasia. (They used unconcentrated urine and failed to relate the time of collection of the sample to the time of the bleeding.)

Since the studies of Hartman, Firor and Geiling<sup>5</sup> called attention to the fact that a factor from the anterior lobe of the pituitary gland might be concerned as a direct etiologic agent in the induction of uterine bleeding, several investigators have studied the blood and urine of normally menstruating women for the presence of a hormone or hormones of the anterior lobe of the hypophysis. Frank, Goldberger and Spielman<sup>56</sup> demonstrated that the ovarian stimulating factor or factors of the anterior lobe may be found in the circulating blood of normally menstruating women. They found the greatest amounts during the early period of the cycle from the sixth to the ninth day following the onset of the previous menstruation. Neumann and Peter,<sup>59</sup> on the other hand,

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57. Zondek, B.: *Zentralbl. f. Gynäk.* **54**:1, 1930.

58. Frank, R. T.; Goldberger, M. A., and Spielman, F.: *Proc. Soc. Exper. Biol. & Med.* **28**:999, 1931.

59. Neumann, H. O., and Peter, F.: *Klin. Wchnschr.* **10**:2086, 1931.

found the hormone from the anterior lobe most abundant during the premenstrual phase. The factor from the anterior lobe has also been found in the urine of normal women. Zondek<sup>60</sup> found a maximum secretion of this hormone just before menstruation; there was a fall directly after menstruation. Katzman and Doisy,<sup>61</sup> studying the urine of normally menstruating women for the presence of the hormone from the anterior lobe found that urinary excretion was high at the time of ovulation and during menstruation.

The studies cited serve as controls for studies concerned in determining the possible presence of hormone or hormones from the anterior lobe in the blood or urine of patients with endometrial hyperplasia. Fluhmann<sup>23b</sup> examined the blood of ten patients with endometrial hyperplasia; only one showed the presence of the hormone. Mazer and Goldstein<sup>40</sup> were unable to find the hormone of the anterior lobe in the blood of eight young women with functional menorrhagia. (No endometrial observations were made.) Fluhmann<sup>23c</sup> described a group of patients which he termed "hypohormonal." This group consisted of young women with polymenorrhea and women near the menopause exhibiting too profuse and too frequent menses. These patients showed pituitary factors from the anterior lobe in their blood. Although such patients may show endometrial hyperplasia, Fluhmann is of the opinion that this is not generally the rule. It was proved that two of the patients who exhibited hypophyseal factors from the anterior lobe in the blood did not have endometrial hyperplasia. It seems fairly clear from the studies presented that endometrial hyperplasia is not associated with the presence of pituitary factors from the anterior lobe in the blood.

On the other hand, patients who exhibit a definite hypo-estral condition may exhibit large amounts of pituitary factors from the anterior lobe in the blood. Fluhmann<sup>23c</sup> found such factors in the blood of castrated women after the menopause and in cases of amenorrhea of long standing.

Smith and Rock<sup>62</sup> studied the urine of patients with menorrhagia and metrorrhagia. They found that fourteen of eighteen specimens collected during the period of bleeding contained a substance which produced follicular ripening when injected into immature rats. In the nonbleeding groups eleven of thirteen specimens were completely negative.

#### THE TISSUE CHANGES

The changes in the endometrium are the result of an excessive proliferation resulting from an abnormal estrogenic stimulus. In considering these changes it seems essential to show their relation to and their

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60. Zondek, B., quoted by Katzman and Doisy.<sup>61</sup>

61. Katzman, P. A., and Doisy, E. A.: *Proc. Soc. Exper. Biol. & Med.* **30**: 1188, 1933.

62. Smith, G. V., and Rock, John: *Surg., Gynec. & Obst.* **57**:100, 1933.

point of departure from the normal. In describing the condition we shall first trace the endometrium through the postmenstrual and interval phases into the hyperplastic state. In the postmenstrual phase the endometrium is thin and pale. The thickness varies between 0.5 and 0.9 mm., and the surface is smooth. The few slender, cylindric glands expand gradually from the surface and follow a wavy course to the muscularis. The epithelium is composed of low, narrow cylindric cells, the inner borders of which are smooth and well defined. The cytoplasm is scanty, of a homogeneous consistency and acidophilic. Some of the cells have cilia. The nuclei stain deeply, may be round but are usually oval, and occupy a basal position. Mitoses are at first infrequent but increase throughout the postmenstrual and interval phases. There is no sign of secretion in the cells or in the lumens of the glands. The stroma cells are spindle-shaped or oval. The cells in the superficial portions are arranged more loosely than in the deeper. The nuclei stain deeply and almost fill the cell. There are little edema and few polymorphonuclear leukocytes. The glands increase in length and their lumens widen as the postmenstrual phase merges into the interval phase. Both phases are characterized by a proliferation of the endometrial elements as a result of the estrogenic stimulus. The terms are retained only on account of their general use. It would be better if they were eliminated and the term "proliferative phase" or "estrogenic phase" substituted. In the latter half of the proliferative phase (the old interval phase) the glands increase in length and their lumens widen. The glands open through a narrow neck, and they expand basally in a somewhat uniform manner. A rare gland may show no expansion of the lumen. Occasional glands may show cystic or fusiform dilatation while others are oval. The growth of the glands is more rapid than the growth of the stroma, and this produces the characteristic sinuous form. The number of glands is increased, probably by the growth of basal buds from the lamina basalis to the surface. O'Leary,<sup>63</sup> who was the first to mention these structures, described them as follows:

From the portions of the glands surrounded by the denser fibroblastic stroma of the lamina basalis, vertical tubules arise which project for varying distances into the stroma of the functional layer and which have no connection with the surface. However, since they reach a much greater length in older mucous membranes, we may assume that they eventually reach the surface and establish new glands, thus serving to increase the number of glands in the later stages. Mitoses in these structures indicate that they are actively growing.

Late in the proliferative phase some glands are dilated and filled with a coagulated material, which is faintly positive to mucicarmine. This material is not of the same appearance or character as the mucoid

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63. O'Leary, J. L.: *Am. J. Anat.* **43**:389, 1929.

secretion seen in the premenstrual phase, and its relation to the latter is not as yet clear. Bartelmez<sup>64</sup> suggested that there are two phases of secretion in the menstrual cycle, the first characteristic of the early interval, the second characteristic of the premenstruum. During the interval there is little intracellular secretion, and the cells are higher and broader. They are somewhat crowded together, giving an appearance of pseudostratification. The cytoplasm is increased, and the nuclei stain deeply. Mitoses are frequent. The inner cell border may lose its sharp outline and assume a fringed appearance. Glycogen is said to be absent. Superficial edema of the stroma may be present.

In hyperplasia the fundamental tendency is a continuation of growth. It is a growth which exceeds normal bounds. Some specimens show marked evidence of this excessive growth while in others it is less marked. In fact it is often difficult to determine from histologic appearances alone where the normal ends and the abnormal begins. Mack<sup>65</sup> studied the degree of hyperplasia in two hundred specimens and found that it was circumscribed in 15, mild in 24, moderate in 49, strong in 10 and very strong in 2 per cent.

In the mild cases there is an absolute increase in the number of glands and of cells. As a result there is a certain irregularity of pattern that stands out in contrast to the normal. There are minor variations in the course of the glands; some are straight, some saw-toothed, and some sinuous. Occasionally, bizarre forms are seen. Variations in the size of the lumens are apparent; a few cystic glands are scattered throughout the endometrium. In some glands the epithelium is single-layered; in others it is denser and often pseudostratified, so that the epithelium has a thick, massive appearance. Epithelium of all kinds may be found in the cystic glands. Frequently it is flattened. Mitoses are commonly seen in good preparations. The vessels are moderately large and engorged with blood; they extend throughout the whole thickness of the epithelium. The stroma is markedly variable. In some areas it is thick; in others, thin. Mitoses are found rather commonly in the connective tissue cells of the stroma. Edema is commonly observed and varies in different parts of the same section. It is to be looked on as a manifestation of circulatory stasis, and in arriving at a conclusion regarding the state of the specimen the edematous areas should be excluded as abnormal and only the nonedematous areas judged. Under the surface epithelium there may be dilated veins surrounded by an area of edema or hemorrhage. The presence of venous thromboses surrounded by areas of necrotic tissue is an important and characteristic finding.

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64. Bartelmez, G. W.: *Anat. Rec.* **35**:3, 1927.

65. Mack, Harold: *Zentralbl. f. Gynäk.* **53**:2068, 1929.

In severe cases, the size of the glands is more variable. The larger glands are cystic, and some are as large as a low power field. Marked variations in shape are noted. Seen in cross-section some are practically round; others have large scallops fairly regular in size. Still others have rounded or pointed projections into the lumen. Longitudinal sections show similar variations of a less extreme variety. These variations in the size of the gland lumens produce the well known "Swiss cheese" appearance to which Novak called attention. The glandular epithelium is very dense at points. In some areas there are two or more layers of cells. The nuclei of the inner layer of cells are flattened, and those of the outer are round or oval. In other areas there seems to be only one layer of cells, but these are so crowded together that they exhibit a pseudostratified appearance. The location of the nuclei varies; some are central and some are basal. The nuclei are flattened and cigar-shaped, and appear to touch each other. Some are wedge-shaped from pressure. Mitoses are frequently seen in good preparations; in poorly fixed specimens they are difficult to find. The inner cell borders are usually clear and distinct, but sometimes they are ragged and seem to be crumbling away. In many cases the lumens of the glands are absolutely empty; in others they contain a variable amount of coagulated material and cellular debris. Occasional cells with distinct pyknotic nuclei and an abundance of granular cytoplasm are present in the lumens. The stroma undergoes the same variations as seen in the milder cases, except that the capillaries are fuller and the veins more dilated.

The presence of areas of necrosis is of considerable importance. Schroeder,<sup>21b, d</sup> stressed the frequent occurrence of these areas and their importance in the development of bleeding. According to him the primary change in the development of these areas is a stasis of blood in the thin-walled, dilated vessels. This brings about disturbances in the nutrition of the surrounding tissue, resulting in areas of edema, areas of hemorrhage and areas of necrosis. Fluhmann<sup>23a, b</sup> found necrotic areas in a high percentage of cases and, like Schroeder, he considered them of great importance. He quoted the work of Mikulicz Radecki who, by means of a viewing instrument or hysteroscope, noted hemorrhages from isolated vessels and defects in the endometrium. The latter were noted especially in cases of endometrial hyperplasia. Other authorities, notably Novak, have considered the necroses of less importance. Novak<sup>22f</sup> stated:

It has always seemed to me that these areas, often very small, can hardly be looked upon as the source of the often extremely profuse bleeding exhibited by these patients, and that some local biological factor must be present in the endometrium, increasing the permeability of its blood vessels. This seems likely in view of the frequent gross intactness of the mucosa, even when the bleeding has been profuse and prolonged.



In another article<sup>22g</sup> he expressed the following opinion: "One cannot, as a matter of fact, be sure that the necrotic areas are not the result rather than the cause of the bleeding." In our own patients we have frequently noted the occurrence of these areas of necrosis, and we believe that they are in some way connected with the process of bleeding.

#### FACTORS IN THE BLEEDING

The bleeding of the hyperplastic endometrium is due in some instances to factors other than the hyperplasia, as patients have been observed who had a hyperplastic endometrium yet did not exhibit bleeding. In fact, we have recently found an endometrial hyperplasia in the uterus of an 8 months fetus. On the other hand, there are certain distinct anatomic differences between the bleeding processes originating in the normal endometrium and those originating in the hyperplastic endometrium. In normal menstruation the necrosis affects the entire mucous membrane, and the separation of the necrotic layer takes place within from twenty-four to forty-eight hours. After the necrotic area has been cast off, the basalis lies open, and bleeding occurs from the stumps of the vessels. This bleeding from the basal layer is rarely severe and stops within a short while. In the hyperplastic mucous membrane the necrosis occurs in small, widely separated areas and gradually extends so that even two or three months may elapse before desquamation takes place. In fact desquamation of the epithelium down to the basal layer may never occur. In the hyperplastic epithelium, as in the normal, bleeding stops promptly when the endometrium is removed with the curet and the basalis is exposed. It seems, therefore, that the failure of the endometrium to undergo proper desquamation is an important factor in the profuse bleeding. Whether this is due to the great anatomic differences between the normal and the hyperplastic endometrium or to a derangement of some common factor governing desquamation is not clear at present. Graves<sup>35</sup> made the following interesting observation in this connection:

A study of uterine blood in functional metrorrhagia was initiated by making daily examinations of the discharge from a girl of 23 with long standing idiopathic hemorrhage. Later biopsy from curettage revealed a marked gland dysplasia. Microscopic examination failed to show the characteristic endometrial remnants seen in normal menstruation. This single observation taken in conjunction with the nearly universal occurrence of clotting in our cases supports the conclusion otherwise logical, that the blood in dysfunctional hemorrhage is deficient in or entirely lacks the secretory elements that would be evidenced by a normal corpus luteum. In other words, it approaches or may be equivalent to an ordinary body hemorrhage according to the extent of the functional disturbance. This probably explains the severe and uncontrollable hemorrhages that are frequently encountered.

The factor of traumatism as a cause of bleeding has been mentioned by Seitz,<sup>66</sup> and this undoubtedly plays a rôle in cases in which a hyperplastic mucous membrane comes in contact with a submucous fibroid. Whether contact between the endometrium on the anterior and posterior walls plays a rôle has not been mentioned. We have noted this contact in cases of hyperplasia and have seen areas of hemorrhage on both sides at the point of contact. It is also not inconceivable that sudden increases in intra-abdominal pressure may cause a rupture of one of the thin-walled superficial veins. Since the endometrium is directly under the control of the ovaries and the ovaries are under the control of the anterior lobe of the pituitary gland, investigators have looked to these organs for an explanation of the endometrial changes. As previously mentioned, Shaw<sup>27</sup> advanced the opinion that the cause of the disintegration of tissue in the hyperplastic endometrium is a toxin produced in the ovaries. He further held that the disintegration of tissue is almost identical in its histologic features with that occurring during menstruation. Burch and Burch<sup>67</sup> suggested abnormal and irregular declines in estrogenic substance as the causative factor for the long continued bleeding. It is apparent that there must be a point at which the amount of estrogenic hormone ceases to be sufficient to maintain the endometrium. If the amount necessary for maintenance is barely present, it is not inconceivable that necrosis may occur at points of lesser circulation. These points would reasonably be in the periphery of the tissue or close to the surface, and localized instead of generalized necrosis would occur. If the decline in estrogenic hormone should be of long duration and slight degree other areas would be slowly involved, and a process not unlike that seen in hyperplasia would result. Siebke's<sup>68</sup> demonstration that estrogenic substance is present in the blood of patients with hyperplasia in the interval and early stages of bleeding but not in the late stages of bleeding is of especial importance in this connection. The results of Smith and Rock<sup>62</sup> suggest that the hormone, like that from the anterior hypophyseal lobe, which they found in the urine of patients with hyperplasia during the period of bleeding only may be associated with the etiology of the condition. Novak and Hurd<sup>69</sup> expressed the opinion that the bleeding is determined by a bleeding factor which is still unknown, but which appears to be bound up in some way with a disturbance in the balance between the follicle-stimulating hormone from the anterior lobe and the luteinizing hormone.

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66. Seitz, A.: *Arch. f. Gynäk.* **116**:252, 1922.

67. Burch, L. E., and Burch, J. C.: *Am. J. Obst. & Gynec.* **25**:826, 1933.

68. Siebke, H.: *Arch. f. Gynäk.* **146**:417, 1931. Siebke.<sup>53</sup>

69. Novak, E., and Hurd, G. B.: *Am. J. Obst. & Gynec.* **22**:501, 1931.

Fibroid tumors are generally considered to be a common cause of uterine bleeding, and every pathologist has seen many cases in which the fibroid was clearly responsible for the bleeding. In a definite percentage of cases, the tumor is small, subserous or intramural and produces no visible alteration in the endometrium. Nevertheless bleeding is present and is so severe as to demand operation. In many other cases there is a well marked hyperplasia in the endometrium with a definite fibroid in the myometrium. In such instances one is inclined to consider the hyperplasia as the cause of the bleeding and the fibroid as a more or less unimportant accompaniment. Cases of this kind are commonly observed. King <sup>70</sup> in a recent article stated:

Another fact of interest bearing on the clinical side of hyperplasia is its frequent association with fibroid tumors. This is a matter of common observation, but there is as yet no adequate explanation to account for it. Its frequency would surely exclude its being a chance relationship. It is more commonly found in connection with the smaller tumors, as the larger ones by stretching and pressure are likely to produce endometrial atrophy. In a series of 114 fibroids treated by radium, I found that 81, or 71 per cent showed hyperplasia. The practical point in this connection is that bleeding associated with many of these tumors is undoubtedly due to the presence of hyperplasia and not primarily to the fibroid. A further suggestive fact is found in the frequent combination of endometrial and myometrial hyperplasia, and the clinician awaits with interest any forthcoming explanation.

Witherspoon <sup>71</sup> has discussed the rôle of ovarian dysfunction, resulting in follicular cysts, as a possible factor in the causation of fibroids. He reasoned that the abnormal estrogenic stimulus affected not only the endometrium but the myometrium as well. Since fibroids grow slowly, he concluded that an abnormal estrogenic stimulus would produce an immediate hyperplasia and gradually developing fibroids. In a further study Witherspoon and Butler <sup>72</sup> investigated one hundred and twenty-five cases of fibroids in Negro women. The facts derived from their study tend to support Witherspoon's hypothesis. They expressed the opinion that the enormous incidence of pelvic inflammatory disease in the Negro produces hyperplasia as a result of inflammatory changes in and around the ovary. The same stimulus which produces the hyperplasia likewise produces the myometrial growth. Clinically, the association of fibroid and hyperplasia is of extreme importance. Unless it is definitely determined which is responsible for the bleeding, the patient may receive treatment for one and be suffering from the other. Under certain conditions the treatment of the two is essentially different, and if this point is borne in mind many proposed operations will prove unnecessary.

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70. King, James E.: *Am. J. Obst. & Gynec.* **26**:582, 1933.

71. Witherspoon, J. Thornwell: *Surg., Gynec. & Obst.* **56**:1026, 1933.

72. Witherspoon, J. T., and Butler, Virginia: *Surg., Gynec. & Obst.* **58**:57, 1934.

From a practical standpoint the diagnosis of endometrial hyperplasia is of extreme importance; it is not always easy, and even in the best laboratories the percentage of errors is high. Taylor<sup>73</sup> made the following statement concerning the experience at the Roosevelt Hospital:

The work of reviewing the microscopic material had scarcely begun when it became clear that the problem, at least so far as the Roosevelt Hospital was concerned, depended upon the correction of diagnosis by the definition of the morphologic characteristics of glandular hyperplasia and its clear separation from physiologic forms of hypertrophy on the one hand and from early carcinoma on the other.

During the five year period, 1925-1929, the diagnosis of hyperplasia or hypertrophy, or the apparently synonymous terms, hyperplastic or hypertrophic endometritis, were applied to 257 specimens of which, after extensive resectioning of material, 216 were available for satisfactory microscopic review. Of this number only 88 would probably be classed as glandular hyperplasia.

Taylor used the term "glandular hyperplasia" in reference to the endometrial changes which we have described as endometrial hyperplasia. After reviewing our own series, we can confirm his observations. In our opinion the main difficulty has been in the use of the term "hyperplasia." The endometrium is a constantly changing tissue, and it is difficult to define the exact line at which the normal ends and the pathologic begins. In the sense that hyperplasia is an abnormal multiplication of tissue elements, it is apparent that such a process could affect any of the elements of the endometrium at any phase of the cycle. Therefore in reviewing a series of cases we find that the diagnosis has been made because there is a thick stroma, an exceptionally large number of glands or an abnormal multiplication of cells. These changes may have occurred in any of the phases of the cycle and in patients who were menstruating normally as well as in those menstruating abnormally. In the sense in which we have employed the term in this article it is limited to patients exhibiting an abnormal increase in the elements of the endometrium, an absence of functioning corpora lutea and a profuse and irregular hemorrhage.

However, experience has shown that in many cases of pathologic bleeding little increase in the elements of the endometrium can be demonstrated. In some cases in which the specimen has been obtained at the end of a long period of bleeding one finds an endometrium similar to that seen in the interval phase. It is true that it is hyperplastic in the sense that it is further advanced in the cycle than it should be. Yet it is similar to other specimens which, from the morphologic point of view alone, are considered normal. In still other specimens the bleeding originates in an endometrium corresponding in time relations and histology to the type seen in the interval phase. These patients do not

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73. Taylor, H. C. Jr.: *Am. J. Obst. & Gynec.* **23**:309, 1932.

exhibit hyperplasia in the sense of an abnormal multiplication of tissue, yet there is an absence of corpora lutea as evidenced by the absence of progestational changes. While they do not exhibit the advanced morphologic changes of hyperplasia, clinical and experimental facts indicate that the changes shown are different forms of the same process and are to be classified with it. The presence of an estral hyperplasia of the endometrium is therefore not absolutely essential to the diagnosis of "endometrial hyperplasia."

The absence of corpora lutea can be determined with certainty only from an examination of the ovaries. Since the retrogression of the corpus luteum requires several months, an ovary may contain several corpora and yet have none corresponding to the present cycle. A knowledge of the life history of the corpus luteum is therefore essential in determining whether the corpora are retrogressing or not. Nonfunctioning or deficiently functioning corpora are theoretically possible. They occur in the rat and mouse. No data are available on this point in regard to human beings. A failure to function would manifest itself by a continuation of the interval type of endometrium and an absence of progestational changes. In the vast majority of cases the ovaries are not available for pathologic examination, and the presence or absence of functioning corpora must be established by the endometrial changes. If progestational changes are present, functional corpora lutea are present; if progestational changes are absent, functional corpora lutea are absent. These progestational changes affect the whole endometrium but are best observed in the glandular epithelium. The cell surface projecting into the lumen appears ruptured or frayed as if melting away into the mucoid secretion in the lumen of the glands, which has a strong affinity for mucicarmine. The cells are wider and broader than in the interval, and hence the nuclei are farther apart. The increase in the volume of the cell results in projections of the epithelium toward the lumen. In these projections the nuclei appear crowded together while in the nonprojecting portions the nuclei tend to be round and assume a basal position. These round, widely separated, basal nuclei give to the tissue a regular, orderly appearance which stands out in contrast to the piled up, rapidly growing cells of the interval. The cytoplasm loses its staining reaction and diminishes in volume. The compact, spongy and basal layers are well defined. In the compact layer one may occasionally find well developed decidual cells. If these progestational changes are present, one is not dealing with endometrial hyperplasia. Before this diagnosis can be established it is essential that the bleeding originate in an endometrium which reflects only the changes produced by the estrous hormone. We have designated this as an "aluteal endometrium."

The pathologist is, of course, dependent on the clinical history for the facts relating to the abnormal bleeding. Many gynecologists still seem to think that hyperplasia is a constant, definite, morphologic condition with stationary characteristics. They fail to realize that in many cases belonging to the hyperplasia group the endometrium shows little deviation from the normal interval type. The abnormality is not in the changes exhibited by the endometrium but in the lack of conformation to the type from which normal bleeding usually occurs. Excessive bleeding from any type of endometrium is pathologic. Any type of bleeding from an aluteal type is unusual; when excessive it is pathologic, and it is this condition of excessive bleeding originating in an aluteal endometrium which should be designated as endometrial hyperplasia. It is the function of the pathologist to determine the type and characteristics of the endometrium, and it is the function of the clinician to determine the type and character of the abnormal bleeding. Only by taking into account the information obtained by both can the diagnosis be established. When these facts are generally realized and every specimen of endometrium is accompanied by a careful and thorough menstrual history, the diagnosis of endometrial hyperplasia will be simplified and information obtained for further advances in the study of the sex cycle.

#### DIAGNOSIS

Our own studies have shed some light on the difficulties of diagnosis. It soon became apparent that it would be impossible to study hyperplasia in the same patient throughout several weeks if we were to depend on repeated curettings for our material. Moreover, in addition to the necessity for hospitalization and anesthesia, the procedure was found impractical, owing to the resulting disturbance of the cycle and the difficulty of evaluating the rate of regeneration of endometrial tissue. Klingler and Burch<sup>74</sup> developed a method which partially overcame these difficulties. It consisted in applying suction to the uterine mucosa by a cannula inserted through the cervix. The cannula had a simple opening at the end and was of the size of an ordinary uterine sound. Suction was obtained by means of an attached syringe. With this arrangement it was possible to obtain small bits of tissue. The procedure caused little pain and could be carried out in the office or dispensary. By the use of this method we obtained serial specimens of tissue from the same patient. The suction method was not entirely satisfactory, however, and the attention of one of us (Dr. Burch) was directed to the possibility of improving it by the intra-uterine use of the ordinary cup specimen forceps. Owing to the intelligent and skilful

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74. Klingler, H., and Burch, J. C.: *J. A. M. A.* 99:559, 1932.

cooperation of the instrument maker<sup>75</sup> an intra-uterine forceps has been made which is entirely satisfactory. The cup is about the size of the bulbous end of an ordinary uterine sound, and since it is mounted on a flexible shaft, it can be inserted as easily as a sound. The biting out of the endometrium is about as painful as an ordinary pinch. The cup usually holds the material obtained from two bites of the forceps, and at each insertion of the instrument two specimens of tissue from different areas may be obtained. The procedure is carried out in the office or dispensary, and patients have been followed for as long as two or three months. In addition to its usefulness as a means of study, the intra-uterine biopsy method has done much to eliminate the use of the curet as a diagnostic instrument. By taking biopsies at frequent intervals in the course of the disease we have been able to obtain considerable information. Hyperplasia has been noted before, during and after a spell of abnormal bleeding; yet ovulation subsequently took place, the hyperplastic endometrium was transformed into a normal premenstrual endometrium, and a normal menstruation ensued. The presence of hyperplasia does not, therefore, exclude the possibility of a subsequent ovulation, the formation of a premenstrual endometrium and normal menstruation, unless the specimen was obtained at or very near the onset of bleeding. For this reason, we advocate the taking of specimens as near the onset of bleeding as possible. This will do much to eliminate errors in diagnosis.

Grossly the appearance of the hyperplastic endometrium is somewhat characteristic. There are long polyp-like and fungous growths projecting into the cavity. The fungous appearance may suggest carcinoma. Often the tissues show numerous hemorrhages between which are areas of normal endometrium. The thickness may be as much as several centimeters. Careful examination will often reveal the presence of many small, clear vesicles. These are characteristic. In addition there is no infiltration of the muscle. Experienced observers can usually exclude carcinoma by the presence of vesicles and the absence of infiltration. Occasionally this is impossible and the question has to be settled by a microscopic examination.

Since hyperplasia and cancer are both proliferative processes, it is not unnatural that a possible relationship between the two should have been suspected, and it is, in fact, sometimes difficult to distinguish between carcinoma and severe grades of hyperplasia. Cases of the latter, however, are rare and in the majority of them there is not the slightest resemblance to cancerous tissue. Cullen<sup>76</sup> was emphatic on this point. Schroeder<sup>21b</sup> stated that the merging of the condition into malignancy

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75. The Geo. P. Pilling & Son Co., Philadelphia.

76. Cullen, Thomas: *Canad. M. A. J.* **19**:411, 1928.

had never been observed. Novak<sup>22g</sup> was convinced that there is no predisposition to cancer. Hintze,<sup>77</sup> in a clinical follow up of twenty-four cases of hyperplasia, found no development of carcinoma. Mack<sup>65</sup> considered that there is no tendency to the development of carcinoma. Taylor<sup>73</sup> studied this point with particular care and stated:

Whether from a practical standpoint hyperplasia is to be regarded as precancerous and treated as such remains an open question. The relative frequency of hyperplasia undoubtedly indicates that the individual patient with the disease is reasonably safe, nevertheless it appears that when hyperplasia is at all marked, the possibility of a predisposition to the development of cancer should be considered and the case regarded with the same degree of suspicion now bestowed upon the diffuse forms of hyperplasia of the breast epithelium. In patients of the menopause age and older an adequate dose of radium is particularly indicated, certainly as the most efficient method of controlling bleeding, possibly as a prophylactic measure against the development of cancer.

Up to the present time we have noticed no evidence of hyperplasia developing into cancer in our cases. It is undoubtedly a rare occurrence and should have been noted more frequently if it is of causal significance.

Hyperplasia may occur at any time in life. It is decidedly more common at or near the menopause. Fluhmann<sup>23b</sup> found that 50 per cent of his patients were over 35 years of age. Schroeder<sup>21d</sup> gave the following data in regard to the age of occurrence: from 8 to 9 per cent of the patients were from 16 to 20 years of age; from 8 to 9 per cent were from 20 to 37; from 82 to 84 per cent were 37 and over. The greatest frequency was between 41 and 50. Many other authors gave somewhat similar figures. In Fluhmann's<sup>23c</sup> series of seventy-five patients, 53 had had full-term pregnancies preceding the onset of the condition, while fourteen were nulligravidae. Eight had had repeated abortions and never carried a baby to term. In two cases pregnancy occurred after a curettage had demonstrated hyperplasia. Vaginal bleeding is the chief symptom. It is often continuous, and patients are prone to present themselves for treatment during the spells of bleeding. In twenty-six of Shaw's<sup>27</sup> fifty-three cases there was a preceding history of amenorrhea, and most of the other authors noted the occurrence of the disease after a period of amenorrhea. Preceding the onset of continuous bleeding there may have been a long period in which menstrual irregularities and profuse periods were noted. In other cases the periods have been absolutely normal. The bleeding varies in intensity; sometimes the flow is moderate; in other cases it is excessive and frequently alarming. The presence of clots is often noted. During the time between periods of bleeding there is no intermenstrual discharge, unless the patient previously had some condition, such as gonorrhea, producing an intermenstrual discharge. A well developed secondary anemia is

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77. Hintze, O.: *Zentralbl. f. Gynäk.* **53**:2396, 1929.



present in almost all the severe cases. The hemoglobin is frequently below 40 and may be as low as 25 per cent. As a result of the anemia, dyspnea, palpitation, faintness and headache are noted. Cramping or colicky pain accompanying the bleeding is generally absent. It may be present during the passage of clots. A unilateral or bilateral sense of vague pelvic discomfort is sometimes noted in conjunction with ovarian cysts. The discomforts of an associated prolapse are augmented during the bleeding spells.

Any general debilitating condition may precede the onset of the disease. Pregnancy, the removal of ovarian tissue and pelvic inflammatory disease are often noted in the previous history. Several of our cases have occurred following abortions. Constitutional habitus does not seem to play an especially prominent rôle, although one is struck by the frequent occurrence of the disease in obese women with hairy faces.

On pelvic examination the cervix may be slightly bluish; it is generally soft and patulous during the bleeding. The body of the uterus varies in consistency, being sometimes hard and at other times doughy. Often it is symmetrically enlarged. A small cyst, rarely larger than one's fist, may be palpated in the adnexal region.

The condition must be differentiated from cancer, abortion, fibroid and unruptured ectopic pregnancies as well as from luteal polymenorrhea. The patients with cancer of the body of the uterus are usually older and have a foul serous or watery discharge between periods of bleeding. Postmenopausal bleeding is more frequent in cancer than in hyperplasia. Abortion is suspected on account of the preceding period of amenorrhea and the soft, open condition of the cervix. The age of the patient and the absence of cramping pain speak against abortion. A submucous fibroid can produce many of the symptoms of hyperplasia. Dysmenorrhea, the presence of a hard cervix and the tendency to maintain a cyclic character are suggestive. A difficult problem is often raised by the preceding amenorrhea, adnexal mass and slight bleeding suggesting an unruptured ectopic pregnancy in the younger patients. Shaw<sup>27</sup> held that the disease can be diagnosed with a moderate degree of ease if an accurate menstrual history is available. Our experience substantiates the view that a careful history and physical examination will often lead to a correct diagnosis. While the percentage of error is small, the results of error are serious, and we advocate a study of the endometrium before a final diagnosis is arrived at. Curettage is the general method of obtaining tissue. The possibility of overlooking a circumscribed cancer is theoretically less with curettage than with our biopsy method. However, the latter method is so easy and convenient, and the possibility of a circumscribed cancer is so small that we have been

using the method more and more. A single insertion of the instrument can obtain two pieces of tissue at various points in the cavity of the uterus, and, if one elects, the procedure can be repeated many times. It is therefore possible to obtain almost as many different samples of tissue as can be obtained by the use of the curet. Since this method does not require hospitalization and can be carried out on ambulatory patients there is no valid excuse for not confirming the diagnosis by an examination of the tissue.

The prognosis of the disease as regards life is good, although fatal cases have been reported by Plaut<sup>78</sup> and Munzescheimer.<sup>79</sup> Some patients, for no apparent reason, spontaneously recover, while others continue to bleed and require treatment. Pregnancy may occur after a spontaneous recovery, as mentioned by Fluhmann.<sup>23b</sup> In other instances a cessation of the bleeding is followed by long periods of amenorrhea which are permanent in the older age groups.

The disease is particularly amenable to treatment, and cures can be obtained by the use of hormones, radiotherapy, curettage and hysterectomy. Recent experiences with the so-called anterior pituitary hormone obtained from the urine of pregnant women have been most encouraging. Owing to the similarity of action between it and substance from the anterior lobe it was called prolan by its discoverers, Zondek and Aschheim.<sup>80</sup> Collip<sup>81</sup> found a somewhat similar substance in the placenta. The experiments of Reichert, Pencharz, Simpson, Meyer and Evans<sup>82</sup> and of Hill and Parkes<sup>83</sup> have cast some doubt on the hypophyseal origin of the substance, and it is not improbable that it acts by stimulating the hypophysis, which in turn stimulates the ovary. Good results could be explained as due to the rise in estrogenic substance which would cause a regeneration of the endometrium and a cessation of the bleeding. As previously mentioned, there is a good deal of evidence pointing to a decrease of the estrogenic substance in the blood as being responsible for the degenerative changes resulting in bleeding. Owing to the excessive luteinization of the ovaries of experimental animals the substance came to be known generally as the anterior pituitary luteinizing substance, and as a result there was a tendency to attribute good results in human beings to luteinization. In the discussion of Novak and Hurd's<sup>69</sup> article on the results obtained with the so-called

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78. Plaut, M.: *München. med. Wchnschr.* **76**:112, 1929.

79. Munzescheimer, J.: *Zentralbl. f. Gynäk.* **54**:2953, 1930.

80. Aschheim, S., and Zondek, B.: *Klin. Wchnschr.* **7**:1407, 1928. Zondek, B.: *Klin. Wchnschr.* **8**:157, 1929.

81. Collip, J. B.: *Endocrinology* **15**:315, 1931.

82. Reichert, F. L.; Pencharz, R. I.; Simpson, M.; Meyer, K., and Evans, H. M.: *Am. J. Physiol.* **100**:157, 1932.

83. Hill, M., and Parkes, A. S.: *J. Physiol.* **71**:40, 1931.

luteinizing hormone from the anterior pituitary lobe, Geist<sup>84</sup> reported a series of twenty-two women who were treated with this material preoperatively and whose ovaries showed no excessive luteinization. In closing the discussion Novak<sup>85</sup> expressed the belief that the effect of bleeding is due not to the histologic effect but to an effect on some unknown factor causing the bleeding. Klingler and Burch<sup>86</sup> reported a case in which serial biopsies were made. They concluded that the extract may exercise its immediate effect without producing progestational proliferation. We have found it most efficacious if given in doses of 100 rat units daily at the time the patient is seen, if bleeding is present; if there is no bleeding, the same amount is given, starting on the first day of the period and continuing until it stops. Usually the bleeding will stop within a reasonable time following this treatment. The extracts are somewhat unstable, and fresh preparations should be obtained if possible. Scattered reports from abroad indicate good results from commercial extracts of corpus luteum. As yet these extracts are not available in this country. Novak has repeatedly called attention to the rationale of this procedure. Favorable results have also been reported from parathyroid extract-Collip and from insulin and thyroid extracts. In cases in which hormone therapy fails one must turn to the older forms of treatment. Radium and roentgen rays find their greatest field of usefulness near the menopause, and the results are brilliant. By the use of the biopsy method and roentgen rays, hospitalization can be avoided in suitable cases. In very young patients curettage gives relief from the bleeding and furnishes an opportunity to correct any constitutional disturbance which may have been present. It should be repeated several times before a more radical form of treatment is decided on. Some years ago Dr. R. S. Hill,<sup>87</sup> of Montgomery, Ala., advocated injections of a solution of formaldehyde into the cavity of the uterus. He failed to publish his method, and it is therefore relatively unknown. Ochsner and Sullivan<sup>88</sup> have modified Hill's method and have stated:

Instead of injecting the full strength formalin into the cavity, we pack the cavity for two minutes with plain sterile gauze saturated with full strength formalin. This procedure is, we believe, at least as effective as radiation and has none of its objections.

We have had no experience with the method of treatment suggested by Hill, but we intend to try it. After the failure of conservative methods, hysterectomy with retention of one or both ovaries is preferable to large doses of roentgen rays or radium in patients of the age group between puberty and the menopause.

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84. Geist, S. H.: *Am. J. Obst. & Gynec.* **22**:935, 1931.

85. Novak, Emil: *Am. J. Obst. & Gynec.* **22**:935, 1931.

86. Klingler, H. H., and Burch, J. C.: *Am. J. Obst. & Gynec.* **26**:17, 1933.

87. Hill, R. S.: Personal communication to the author.

88. Ochsner, E. H., and Sullivan, J. Kenneth: *Illinois M. J.* **61**:316, 1932.

## Notes and News

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**University News, Promotions, Resignations, Appointments, etc.**—Appointments and changes in title have been made at the University and Bellevue Hospital Medical College in New York as follows: Douglas Symmers, professor of pathology and director of the pathologic laboratory; Irving Graef, assistant professor of pathology; J. A. Klosterman, associate professor of bacteriology; Maurice Brodie, assistant professor of bacteriology.

John R. Schenken has been appointed instructor in pathology in Georgetown University, Washington, D. C.

At the University of Witwatersrand, Johannesburg, South Africa, a professorship in forensic medicine has been established in cooperation with the government in order to secure better medicolegal service. The professor of forensic medicine will act also as the chief district surgeon or medical examiner for Johannesburg.

**Appointments by Medical Fellowship Board.**—At the meeting of the Medical Fellowship Board of the National Research Council, March 31, 1934, the following appointments were made: Ralph W. Barris, neurology; Harold Blumberg, biochemistry; Dean A. Clark, neurology; Jack M. Curtis, biochemistry; Henry S. Dunning, pathology; Harry B. Friedgood, physiology; Benjamin F. Miller, medicine; Myron Prinzmetal, cardiovascular physiology and pathology; Robert Tennant, pathology.

**Society News.**—The officers of the American Society for Experimental Pathology for 1934-1935 are: S. B. Wolbach, president; Oskar Klotz, vice-president; Shields Warren, secretary-treasurer.

E. L. Opie has been elected president of the Society for Experimental Biology and Medicine; P. E. Smith, vice-president; A. J. Goldforb, secretary-treasurer.

The new officers of the American Society for the Control of Cancer are: Burton T. Simpson, president; Henry K. Pancoast, vice-president; J. M. Wainwright, secretary; Calvert Brewer, treasurer; James Ewing, chairman of the board of directors.

The recently elected officers of the American Association for Cancer Research are: Millard C. Marsh, president; E. T. Bell, vice-president; William H. Woglom, 1145 Amsterdam Avenue, New York, secretary-treasurer.

The newly elected officers of the American Association of Immunologists are: Francis G. Blake, president; A. F. Coca, secretary and treasurer. The next meeting will be held at Cornell University Medical College, New York, on April 17 and 18, 1935.

At its recent meeting in Toronto the American and Canadian Section of the International Association of Medical Museums elected William Boyd president, Virgil H. Cornell vice-president and Maude E. Abbott, McGill University, Montreal, secretary-treasurer.

T. M. Rivers and J. H. Northrop, members of the Rockefeller Institute for Medical Research, have been elected to membership in the National Academy of Sciences.

**American Type Culture Collection.**—In 1911 a Bacteriological Collection and Bureau for the Distribution of Bacterial Cultures was established at the American Museum of Natural History in New York City with C. E. A. Winslow as curator. Its catalog, issued in 1912, contained 350 species. In 1922 this collection was placed in the care of the Army Medical Museum in Washington. In 1925, through the urging of the Division of Medical Sciences of the National Research Council, the General Education Board made an appropriation of \$24,000 for the maintenance of the collection, to be paid over a period of five years. At this time 175 cultures, which represented what remained of the collection, were

transferred to Chicago as the nucleus of the American Type Culture Collection. The collection was placed in the care of the John McCormick Institute for Infectious Diseases, under the direction of a committee representing the Society of American Bacteriologists, the American Phyto-Pathological Society, the American Zoological Society and the American Association of Pathologists and Bacteriologists. The expense of maintaining and developing the collection has been provided by the grant of \$24,000 from the General Education Board covering five years, a grant of \$10,000 made in 1930 by the Rockefeller Foundation, an annual contribution of \$400 from the Society of American Bacteriologists, a few contributions from commercial sources and the income from the sale of cultures. At first \$1 was charged for cultures, but in 1931, when the support from the General Education Board terminated, the price was raised to \$2 in order to cover expenses. From 1925 to 1933, 36,294 cultures were supplied to teaching institutions and others. The collection in Chicago now contains about 1,300 cultures. The preservation of the collection on the present scale is dependent almost entirely on the income from the sale of cultures. General support of the collection is earnestly requested. Those who describe new species are urged to deposit them with the collection. The address of the collection is 629 South Wood Street, Chicago.

**Doctor of Medical Science.**—The degree of Doctor of Medical Science has been authorized for graduate study by Columbia University on the ground that such a degree is needed to identify specially trained physicians. Three years' training after internship with original graduate medical work will be required of candidates for this new degree.

## Obituaries

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### WILLIAM HENRY WELCH 1850-1934

The death of Dr. Welch on April 30, after a long though relatively painless illness that confined him to the hospital for fourteen months, leaves medicine in America deprived of one who was perhaps its greatest figure. No other person has contributed so much toward the development of medical education with the introduction of a new attitude toward medicine. His work in pathology and bacteriology was followed by a broader new conception of the organization of public health control and the establishment of a new school of hygiene and public health. And, as though this were not enough for one man, in his later years he aroused an intense interest in the history of medicine and founded the present institute devoted to that subject. But these three lines of activity which, as others have said, represent three periods in his career, are after all of less significance to this country and to the world in general than the extraordinary influence of his personality and his recognized wisdom, which made him sought out by all great foundations and governmental bodies for his advice in their undertakings. He was president at one time or other of most of the prominent scientific and medical associations and of the boards of scientific direction of such centers of research as the Rockefeller Institute, and at home he was the constant ideal last source of counsel and wise decision. In problems of research or any new plan, the question was always, "What will Dr. Welch think of it?"

Dr. Welch was born in Norfolk, Conn., on April 8, 1850, the son of an able and gifted physician, Dr. William Wickham Welch. After graduating at Yale and later in medicine at the College of Physicians and Surgeons of Columbia, he was an intern at Bellevue Hospital. In 1876, he went to Europe, where he worked in histology with Waldeyer and in chemistry with Hoppe Seyler at Strasbourg, and later with Wagner at Leipzig, and with Ludwig. Ludwig advised him to go to Cohnheim in Breslau, and, after a particularly stimulating semester there, where he completed his study of pulmonary edema, he visited Klebs in Prague and Chiari and others in Vienna.

When he came back to New York he was, after an interval, established in Bellevue Hospital Medical College, where he was given a laboratory and abundant material for the study of pathology. In Europe, he had been fortunate in being associated with such fellow students as Weigert, Ehrlich, Salomonsen and many others, and in New York he

had the fellowship of Prudden and the friendship of such men as Alonzo Clark, Delafield and the older Flint.

For six years he worked in New York, attracting eager students to his autopsy demonstrations and laboratory work. Then came his appointment to the professorship of pathology in the Johns Hopkins University in 1884. He spent that year in Europe, immersed in the new discoveries of Koch, Pasteur, Pettenkofer and others. As Tomlinson said, "Could Sinai itself impose its revelation on a climber, who was no Moses?" but though others in his time had gone in numbers to Europe to study, he brought back the spirit and impulse that stirred America to the enormous effort that has changed the whole of medicine.

He returned in 1885 to Baltimore to begin work with a group of enthusiastic assistants, among whom were Councilman, Halsted, Mall, A. C. Abbott, Reed and Nuttall. The years until the opening of the medical school in 1893 were most productive, and Dr. Welch to the last loved to recall "that little group" with whom he plunged into the new bacteriology and immunity. Flexner arrived just before the medical school opened and remained until 1899, during which time he was much associated with Dr. Welch in studies of diphtheria and other subjects.

The influence of Dr. Welch in the choice of the first faculty of the School of Medicine seems to have been paramount, and there can be no doubt of the happiness of that selection. Osler, Halsted, Kelly, Mall and Abel were there, and during those years there was always a feeling in the school that any troublesome problem could be referred to Dr. Welch, with confidence in the wisdom of his reply. His lectures on pathology were most stimulating and suggestive, and his whole attitude stirred his assistants to great effort.

In 1917 he resigned from the chair of pathology and devoted himself to the broad problems of public health, with the new School of Hygiene as his headquarters. During that time, as always, his advice was sought on all sides. With the completion of each new institution for medical research, it was hoped that he would give the opening address, and he gave many of these, which were always inspiring to the workers in those places. His association with the varied activities of the Rockefeller Foundation took him twice to China, especially in connection with the newly founded Peking Union Medical College, and on other trips to Europe he was the honored guest or speaker on many occasions. In 1926, he resigned from the directorship of the School of Hygiene in order to devote himself to the history of medicine, for which a new foundation was set up. He went to Europe and everywhere sought out, and, with profound knowledge, chose books for the new institute. While he was away the Welch Library, which was to house the Institute of the History of Medicine, was planned and built, and on his return it was

opened with suitable ceremony. The photograph shown here was taken on this occasion in October 1929. Dr. Welch became emeritus professor of the history of medicine in 1931.

His writings are characterized by a remarkable clearness of style and a really extraordinary wealth of knowledge of the literature. They are long and detailed, but make delightful reading. His was a mind open to every source of information, completely free from prejudice and judicial in type, so that he carefully weighed statements on one side or the other and expressed his opinion after this precise balancing of the evidence.



WILLIAM HENRY WELCH, M.D.  
1850-1934

His most important contribution to the facts of medicine are perhaps his discovery of the gas bacillus and his studies in diphtheria, hog cholera and the structure of thrombi, but his splendid papers on thrombosis and embolism and on fever and other subjects are of lasting value. Then, too, his expression in various papers and addresses of his ideas concerning medical education, public health and the organization of institutes of research will always be treasured and studied by medical men and those responsible for medical advances in the future.

He was, as every one knows, a most lovable character, entirely free from any undue sense of his own importance, so that he was accessible to all and so friendly that probably much of his time was occupied by



constant visitors. His friends were innumerable, and he was often the generous host at dinner at the Maryland Club. In the early days he spent much time there with a small group, including Dr. Halsted, Major Venable and Mr. Hambleton, in spirited conversation in a small room which has ever since somehow retained the memory of those meetings. They were epicures, too, Dr. Welch perhaps the most discriminating of all, and there was some truth in the New York prophecy that in Baltimore he would become an epicurean judge of terrapin and madeira. Later, he was for many years until his death president of the University Club and spent much time in its excellent library.

His memory was phenomenal, and no last detail seemed ever to fade out, although he read constantly such works as the "Encyclopaedia Britannica" and the "Dictionary of National Biography." Nor were his own experiences through a long life any more evanescent, for he could at any time recount in detail the happenings of his school days or of a long past stay in Europe. His command of medical literature, whether relating to pathology or to the history of medicine, was remarkable, too, and he gave many astonishing revelations of it.

He was, of course, honored in many countries by their universities, which conferred degrees or medals or some other distinction. On the twenty-fifth anniversary of his doctorate, a great volume of papers was *published in his honor, and on his eightieth birthday every country vied with America to do him honor.* His face is perpetuated in the Sargent portrait and in other portraits, notably one by Corner; a bronze bust stands in the Welch Library, and there is a moving talking picture in which he is seen recounting the experiences of his life.

It was a very perfect life, of great significance to the world, and on the whole intensely interesting and happy for himself. There was nothing to regret at any point, and his intellect and wisdom and remarkable sympathetically controlling personality were expressed to the full.

The organization of education in medicine, public health and the history of medicine owes its origin and progress largely to Dr. Welch's efforts and wisdom, and, beside that, his personal influence has been felt through all these years as a compelling inspiration to his many students who have carried his ideas all over the world.

His death is an irreparable loss, but his memory will not fade and will continue forever its stirring influence transmitted by those who knew him.

WILLIAM G. MACCALLUM, M.D.

# Abstracts from Current Literature

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## Pathologic Anatomy

ATRESIA OF THE TRICUSPID ORIFICE. GEORGE R. MURPHY and LEO F. BLEYER, *Am. J. Dis. Child.* **46**:350, 1933.

A case is reported with postmortem observations of an infant about 4 months old showing congenital atresia of the tricuspid orifice with an interventricular septal defect, a patent foramen ovale and a rudimentary right ventricle. In addition to the cardiac condition, there was diffuse bronchopneumonic solidification, with delayed resolution and beginning cyanosis and dyspnea. If one includes the case of Cathala and Tisserand, this case is about the fifteenth reported showing this anomaly of the heart.

AUTHORS' SUMMARY.

CONGENITAL ABSENCE OF GALLBLADDER AND EXTRAHEPATIC DUCTS. J. MONTGOMERY DEEVER, *Am. J. Dis. Child.* **46**:356, 1933.

A white girl presented no abnormality until 6 weeks of age, when there began to be noticeable icterus. The icterus varied in intensity from day to day; the stools were clay-colored, and the urine was dark. There was a tendency toward constipation, and the child did not gain normally in weight. Repeated examinations of the stools and urinalyses gave negative results for bile pigment. The icteric index was 60, and the fragility test gave normal results. The Wassermann and Kahn tests were negative. The child died at the age of 1 year and 12 days. At necropsy the gallbladder could not be found, and careful dissection failed to reveal evidence of extrahepatic ducts. Both macroscopically and microscopically the liver showed marked biliary cirrhosis, with proliferation of the intrahepatic ducts. The papilla of Vater was absent. The spleen showed passive congestion, fibrosis of the pulp and hemosiderin pigmentation.

RALPH FULLER.

FETAL ENDOMYOCARDITIS: INTRAUTERINE INFECTION AS THE CAUSE OF CONGENITAL CARDIAC ANOMALIES. SIDNEY FARBER and JOHN HUBBARD, *Am. J. M. Sc.* **186**:705, 1933.

Fetal endomyocarditis as one of the causes of congenital heart disease is discussed. A summary is given of ten cases recorded in the literature and of four cases observed by the authors. The patients were all free from congenital syphilis. In each of the fourteen cases there was gross as well as microscopic evidence of old infection. In view of the early death of each infant it appears that the infectious process must have occurred during intra-uterine life. The nature of the infection is uncertain.

FROM THE AUTHORS' SUMMARY.

STRUCTURE AND FUNCTION OF FILAMENTS PRODUCED BY LIVING RED CORPUSCLES. JOHN AUER, *Am. J. M. Sc.* **186**:776, 1933.

The living red corpuscle of the guinea-pig, rabbit and dog and of man in hanging-drop preparations produces from one to five almost colorless, tapering, elastic filaments varying in length from 5 to 75 microns. Filaments may apparently arise from platelets. The filaments may appear within a few minutes after a hanging drop is made and may be readily detectable for more than twenty-four hours. They are composed of twisted fibrils, which in turn are composed of separately invisible subfibrils. The filament, fibril and subfibril are not sticky. The filament is motile and rotates around its long axis to the right and to the left; when one direction predominates, this ultimately causes a shortening. The

filaments anchor themselves peripherally to other red corpuscles, blood shadows, platelets or the edge of the drop. Their shortening distorts and compresses the red corpuscles into various shapes and may produce a microcyte with dense hemoglobin content. The filament may tear peripherally and roll up into a ball, change into a chain of refractile beads or thicken into a refractile rod. All these changes are reversible. The beads and the filament are not doubly refractile; both may appear free in the serum and execute active movements. Single free beads resemble blood dust, but are more lively at first. The formation of filaments is decreased or abolished by sufficient amounts of sodium oxalate, hirudin and heparin, but the formation of filaments is more resistant to these substances than the formation of fibrin from plasma fibrinogen. These filaments are probably the agents that cause the deformation of cells in sickle cell anemia. No contraction or intrinsic movement of typical fibrin threads from plasma fibrinogen was ever seen.

AUTHOR'S SUMMARY.

CARDIAC WEIGHTS. VICTOR LEVINE and JAMES G. CARR, *Arch. Int. Med.* **52**:429, 1933.

Sixty-four human hearts were divided and weighed according to the method described by Herrmann. These included normal hearts, hearts from persons who had died of some form of wasting disease, hypertensive hearts, hearts from persons who had died of rheumatic disease and hearts from persons who had died of cardiovascular syphilis, with and without involvement of the aortic valves. In patients dying of wasting disease the weight of the heart decreases slightly less than that of the body in general. In patients with rheumatic deformity of the mitral valve alone the increase in the weight of the heart is due to an increase in the weight of the right ventricle and of the auricles. In large hearts of persons suffering from rheumatism or syphilis with regurgitation at the aortic valves, the entire heart hypertrophies, but the greatest increase is in the left ventricle. The assumption that the left ventricle alone shows definite hypertrophy in cases of syphilitic aortic regurgitation of some duration is not acceptable. In the production of cardiac hypertrophy mechanical factors are not solely effective. The figures for the large hearts of persons with syphilis show diffuse cardiac hypertrophy whether or not aortic regurgitation was present. The size of hypertensive hearts without valvular disease does not equal that of hearts in which long-standing valvular defects are found at the aortic orifice. In the hypertensive type, however, the proportionate increase in the size of the left ventricle is greater than in other groups. A comparison of our work with that of Herrmann shows that the left ventricular weight right ventricular weight quotient of man is greater than that of the dog. The total ventricular weight/body weight quotient is twice as great for the dog as for man. The customary method of determining the thickness of the ventricular walls at autopsy is apt to be misleading as to the presence of right ventricular hypertrophy, which may be overlooked. The results obtained by the method that we employed and those obtained by Lewis with his method are similar for normal hearts and hearts with syphilitic lesions; they are approximately alike for hearts with rheumatic valvular deformity. In hypertensive hearts and the hearts of persons suffering from nephritis the results obtained by the two methods do not agree.

AUTHORS' SUMMARY.

XANTHOMATOSIS AND THE CENTRAL NERVOUS SYSTEM (SCHÜLLER-CHRISTIAN SYNDROME). CHARLES DAVISON, *Arch. Neurol. & Psychiat.* **30**:75, 1933.

Davison reports the forty-ninth case of xanthomatosis, in a man, aged 29: osseous defects, especially of the cranium; falling out of teeth; diabetes insipidus, and an increase in total fat, but absence of exophthalmos. The cholesterol content of the blood was normal. The anatomic changes were mainly plaques in the white substance of the brain, cerebellum, pons, medulla and basal ganglions. They appeared as areas of demyelination which also contained fat granule bodies filled

with lipoids and were associated with marked reactive glial phenomena—the formation of large cytoplasmic glia cells. Around the tuber cinereum and in the visceral organs were large deposits of lipoids. The ganglion cells were, as a rule, preserved, but were destroyed in the tuber cinereum, which was sclerosed and fibrosed. Davison like other investigators considers the condition a lipoid (cholesterol) disturbance which resembles other conditions of disturbances of lipoid metabolism—Gaucher's disease, Niemann-Pick's disease and amaurotic family idiocy.

G. B. HASSIN.

MEGALENCEPHALY WITH DIFFUSE GLIOBLASTOMATOSIS OF THE BRAIN STEM AND THE CEREBELLUM. ARTHUR WEIL, Arch. Neurol. & Psychiat. 30:795, 1933.

Diffuse glioblastomatosis is regarded as a hyperplasia of glial elements throughout the brain. There may also be an increase in the size of the nerve elements.

A boy who appeared normal at birth had a large head. At the age of 6 years and 4 months he showed apathy and a staggering gait, morning vomiting, drooling of saliva, slurring speech, exaggerated reflexes and the Babinski sign. The condition grew progressively worse and death occurred following bronchopneumonia.

The weight of the brain was 1,856 Gm.; the number of ganglion cells was apparently reduced without increase in the number of glia nuclei; there was a mild vascular infiltration in the white substance. In the diencephalon and midbrain the glia nuclei were increased in number, while the pons, the medulla, the roots of the cranial nerves and the cerebellum were densely infiltrated by glia cells. The ganglion cells were preserved. The pons, medulla, midbrain and thalamus were increased in size, with glioblastomatosis, but the enlargement of the cerebral hemispheres and corpora striata could not be explained by this neoplastic process. The frontal cortex was increased in thickness 45 per cent and the temporal cortex 30 per cent, but the number of ganglion cells was not increased. The hypertrophy of the cortex and corpora striata was due to isolated hypertrophy of the neuroglia, while the glioblastomatosis was confined to the brain stem, cerebellum and upper part of the cervical spinal cord. Glioblastomatosis is a diffuse process which begins simultaneously in different regions of the central nervous system. It differs from diffuse gliosis, in which there is hyperplasia of adult normal glia cells.

G. B. HASSIN.

IMPACTION OF A NEURO-EPITHELIAL CYST IN THE THIRD VENTRICLE OF THE BRAIN. CARL O. RINDER and PAUL R. CANNON, Arch. Neurol. & Psychiat. 30:880, 1933.

A cystic tumor of the third ventricle is described in a woman who complained of acute dimness of vision, severe headache, projectile vomiting and severe pain over the right eye and in the temporal region. A few hours later she became comatose and died. There was an almost complete occlusion of the third ventricle by a cyst attached by a stalk to the anterior margin of the right choroid plexus. The contents were gelatinous, and the wall had an outer fibrous coat and an inner layer of cuboid and columnar ciliated epithelial cells. Some of the latter contained blue or yellowish-brown granules. The authors prefer the term "neuro-epithelial cyst" to "colloid cyst."

G. B. HASSIN.

CONTRACTED KIDNEY DUE TO PYELONEPHRITIS. W. T. LONGCOPE and W. L. WINKENWERDER, Bull. Johns Hopkins Hosp. 53:255, 1933.

Nine patients have recently come under observation, five of whom have died in uremia and have been examined at autopsy. Most of the patients were women, and the disease in each case was probably of long duration. The course in some cases was characterized by attacks of pyuria accompanied by pain in the lumbar region and sometimes by fever. In some patients the course was practically symptomless until uremia suddenly appeared. In five of the nine cases there was

hypertension, while in four the hypertension was combined with hemorrhagic retinitis and arteriosclerosis of the retinal vessels. In the cases of hypertension there was rarely edema unless cardiac failure occurred. In all the patients dry skin, progressive anemia and polyuria were present. There were no evidences of cystitis, except in one patient. Pyelograms showed irregularities of various types in the size and shape of the kidneys, pelvis and calices. The specific gravity of the urine was constantly low, and the amount of albumin was small. Casts were rarely present; red blood cells were scarce or absent, except in one patient with ulcers of the bladder. Evidence of impairment of renal function appeared sometimes years before death and occasionally progressed to a remarkable degree without serious symptoms. Death came in uremia. At autopsy the kidneys were reduced in size, extremely uneven, scarred and distorted and showed irregular, slightly dilated pelves, which presented varying degrees of inflammation. Microscopically, areas of inflammation or retracted scars running from the cortex replaced large portions of renal tissue. Between these areas the comparatively normal kidney tissue was irregularly disposed. There was no gross obstruction to the flow of urine. It is not clear whether the disease arises from bilateral hematogenous infection of the kidneys or from an ascending infection from the bladder. At the time of writing treatment was being directed toward obtaining adequate drainage of the pelvis of the kidney.

FROM THE AUTHORS' SUMMARY.

LYMPHOID TISSUE IN THE SUBSTANCE OF THE KIDNEY. J. W. S. BLACKLOCK, *J. Path. & Bact.* **37**:504, 1933.

Foci of lymphoid tissue containing perfectly formed lymph follicles with germ centers were present in the cortex and, to a less extent, in the medulla of a kidney which was the seat of multiple subacute abscesses and inflammatory infiltration following scarlet fever.

PATHOLOGIC HISTOLOGY OF THE OMENTUM. P. JEBEL, *Beitr. z. path. Anat. u. z. allg. Path.* **91**:441, 1933.

The occurrence of metastases of gastric carcinoma in the omentum and the possible relation of such metastases to lymphoid tissue led to a histologic examination of the omentum in eighty-four cases which came to necropsy in persons of various ages from childhood to old age. The perivascular cellular accumulations that form the milky patches of the omentum of the new-born infant and of lower animals were not seen. The lobules of fat on the surface and margin of the omentum are bounded by a fine fringe of connective tissue, by means of which the omentum becomes attached to surrounding structures when acutely inflamed. Lymphoid follicles are present in the gastrocolic ligament at its attachment to the greater curvature of the stomach, but none was found in the omentum itself. Although metastasis of tumors to the omentum by way of the blood stream is possible, metastasis occurs usually by implantation and not by retrograde lymphatic transport of tumor cells. Atrophy of the omentum in cachectic states is due to disappearance of fat; the connective tissue framework becomes relatively prominent and may form whitish patches. Chronic inflammation of the omentum is associated with the presence of fibroblasts, histiocytes, lymphocytes and plasma cells, but lymphoid follicles are not formed. Leukocytic infiltration in acute inflammation is due to emigration of leukocytes from the vessels of the omentum.

O. T. SCHULTZ.

RHINOSCLEROMA AT NECROPSY. W. NOWICKI, *Beitr. z. path. Anat. u. z. allg. Path.* **91**:457, 1933.

Although rhinoscleroma is a common disease in the portion of Poland in which Lemberg is situated, few persons with this disease are seen on the necropsy table, evidently because the disease is rarely the immediate cause of death or of a terminal

illness that leads to hospitalization. In Nowicki's institute at Lemberg 23 cases of rhinoscleroma were encountered at necropsy within thirty-six years in a total of 31,962 necropsies. The disease may be the cause of sudden death through asphyxia or through hemorrhage complicating tracheotomy. Nowicki included in his series 8 cases of this type from the local medicolegal institute. Males and females were equally affected. Most of the cases occurred in young persons; 5 occurred in the age period of from 11 to 20 years, and 17 in the decade of from 21 to 30 years. The process was not limited to any particular portion of the upper respiratory tract. The larynx was most often involved, but usually in association with the nose, trachea and primary bronchi. Four anatomic forms are described: nodose, tuberosus, diffuse and cicatricial. The essential features of the 31 cases are presented in tabular form.

O. T. SCHULTZ.

INVOLVEMENT OF THE APPENDIX IN THE PRODROMAL STAGE OF MEASLES.  
W. FISCHER, Beitr. z. path. Anat. u. z. allg. Path. **91**:475, 1933.

This is another of the rare instances in which the diagnosis of acute appendicitis during the prodromal stage of measles furnished the pathologist an interesting specimen for histologic study. The lymphoid follicles contained numbers of the peculiar giant cells first described independently by Warthin and by Finkeldey as present in the tonsils in measles and by Finkeldey as present in the appendix. Fischer suggests the histologic examination of Koplik's spots [Davidsohn, I., and Mora, J. M.: Appendicitis in Measles, ARCH. PATH. **14**:757, 1932].

O. T. SCHULTZ.

REDUPLICATION OF THE RIGHT AURICLE OF THE HEART; UNUSUAL CHANGES  
IN THE PULMONIC ARTERIES ASSOCIATED WITH PATENT FORAMEN OVALE.  
H. GOMBERT, Beitr. z. path. Anat. u. z. allg. Path. **91**:483, 1933.

Reduplication of a cardiac atrium unassociated with other gross malformations of the heart is a rare anomaly. Gombert could collect only fifteen instances from the literature. In fourteen the left auricle was double; in only one other case was there reduplication of the right auricle similar to the condition described. Both cases occurred in adults, Gombert's patient being a woman, aged 42. The left auricle was enlarged, and its wall was hypertrophied. The right auricle appeared small externally. It consisted of two cavities, each the size of a walnut. The superior vena cava emptied into the middle auricle. The inferior vena cava opened into the lateral portion of the duplicated right auricle, but delivered most of its blood into the left auricle through a large foramen ovale. The two parts of the right auricle communicated with each other by a small opening through the septum that separated the two parts. The anomaly is ascribed to a failure of union of the primitive auricular septum. In another case, also in a woman, aged 42, an open foramen ovale and hypertrophied right side of the heart were associated with peculiar changes in the terminal branches of the pulmonic artery in the lungs. A proliferation of the endothelium at the point of division of the small arteries acted as a sort of valve. Beyond this point the vessel was tortuous and angioma-like. The condition is ascribed to the altered mechanics of the pulmonary circulation.

O. T. SCHULTZ.

REACTION OF SUBCUTANEOUS CONNECTIVE TISSUE TO INJECTED FINELY DIVIDED  
STONE DUST. E. BENECKE, Beitr. z. path. Anat. u. z. allg. Path. **91**:503, 1933.

For the experimental work reported finely divided stone dust was used. It contained 56 per cent silicic acid and 18 per cent iron oxide. More than 70 per cent of the particles were 5 microns or less in diameter. Rabbits, guinea-pigs and rats were used. A 20 per cent suspension of sterile dust was injected subcutaneously or intraperitoneally. The dose varied from 3 to 6 cc., depending on the species of animal and the route of injection. There were no changes in the tissues that could

be ascribed to the chemical activity of the injected material. The only reaction was the formation of a foreign body granuloma. In a third of the rats there was noted necrosis with softening of the centers of the subcutaneous nodules, similar to what occurs in silicotic nodules of the human lung. Intraperitoneal injection led to the formation of silicotic granuloma in the spleen and liver in two rats, similar to the change noted in these organs in some cases of human silicosis.

O. T. SCHULTZ.

ATHEROSCLEROSIS OF CEREBRAL ARTERIES. K. WOLKOFF, Beitr. z. path. Anat. u. z. allg. Path. **91**:515, 1933.

Wolkoff reports the results of a histologic study of normal and pathologic cerebral arteries in eighty-nine necropsies of persons ranging in age from 2 to 81 years. The pathologic study relates to atherosclerosis, which is compared with the same process in other arteries. Since the work was done in Anitschkow's institute, infiltration with lipoid is accepted as the primary process in atherosclerosis. This process in the cerebral arteries differs in none of its essential features from that in the aorta and the coronary arteries. Infiltration with lipoid occurs in previously normal vessels and is usually localized at the mouths of the larger arteries at the base of the brain or at the origin of their side branches or in the more convoluted portions of the vessels. At these points the intima is normally thicker than elsewhere. Marked atherosclerosis of the larger arteries was occasionally associated with diffuse infiltration of their smaller branches with lipoid. As compared with the aorta and coronary arteries minor differences were the later development of atherosclerosis in the cerebral arteries, the lesser degree of calcification and the slight evidence of regressive or reparative changes.

O. T. SCHULTZ.

CHANGES IN THE BLOOD DUE TO XYLENE. MARIA FÄRBER, Beitr. z. path. Anat. u. z. allg. Path. **91**:554, 1933.

This is a repetition of some earlier work of Woronow, who claimed that derivatives of benzene with a methyl grouping cause changes in the blood of rabbits similar to those of leukemia. In Färber's experiments, 1.5 cc. of xylene in an equal quantity of olive oil was injected subcutaneously into rabbits twice daily. Changes similar to those noted by Woronow resulted. These were hyperleukocytosis, a shift to the left in the leukocytic formula, the appearance of monocytes and of degenerated and young cells of the granulocytic series and hyperplasia of the bone marrow. Färber found the hyperleukocytosis to be transient. Since this change and the shift to the left did not occur when rabbits were subjected to inhalation of xylene, she concludes that these alterations were the result of the local irritating and inflammatory action of the injected xylene. The degenerative changes in the leukocytes were the result of the toxic action of xylene; they occurred after both the injection and the inhalation of the substance. They are not specific for the methyl grouping of the compound, and the altered blood picture cannot be homologized with that of leukemia.

O. T. SCHULTZ.

INFLUENCE OF CEREBRAL DEVELOPMENT ON THE FACIAL SKELETON. E. HAMMER, Beitr. z. path. Anat. u. z. allg. Path. **91**:570, 1933.

Conclusions relative to the influence of development of the brain on the skeleton of the face are drawn from a study of fetal anomalies in which cerebral development was defective. The anomalies studied were divided into two main groups, holocrania and hemicrania. The former was subdivided into four groups of craniorachischisis totalis, with varying degrees of development of the brain. The cases of hemicrania were subdivided into those with and those without closure of the posterior arch of the foramen magnum and with varying degrees of cerebral development. The skeleton of the base of the skull and of the face was found

to have been laid down completely and therefore is initiated independently of the early development of the neural tube. But the further development of the base of the skull is directly influenced by the growth of the brain. Flattening of the primitive occipital bone, resulting from defective development of the brain and failure of closure of the cranial vault, affects the growth of the base of the skull and of the skeleton of the face. The effect on the latter is manifested chiefly in prognathism, abnormal width and height of the nose, malformation of the maxilla and shallowness of the orbits. The effect of congenital hydrocephalus, in which there is excessive action of those factors dependent on the size of the brain and the cranial vault, is contrasted with the effects of defective cerebral and cranial development.

O. T. SCHULTZ.

LOCALIZATION OF RHEUMATIC LESIONS IN THE HEART. M. M. LIEBER, Beitr. z. path. Anat. u. z. allg. Path. **91**:594, 1933.

Detailed histologic examination of the heart in five cases of active rheumatic infection confirmed the findings of others of the frequent localization of the lesions not only in the valvular tissues but in the coronary arteries, the root of the aorta and pulmonary artery, and the auricular endocardium, especially that of the left ventricle. The lesions were granulomatous and were histologically specific, as determined by control examinations of nonrheumatic hearts. Thrombotic deposit on the surface of the endocardial lesions is a secondary phenomenon, many endocardial lesions being devoid of such a deposit.

O. T. SCHULTZ.

THE MENSTRUAL CYCLE AND THE LIPOID CONTENT OF THE UTERINE MUCOUS MEMBRANE. INGEBORG GOHLISCH, Monatschr. f. Geburtsh. u. Gynäk. **94**:223, 1933.

One hundred specimens of uterine mucous membrane were examined for lipoids. Of these, twenty-two were taken in the interval between menstrual periods, seventeen at the beginning of the premenstrual period, thirty-five during the premenstrual period, four during the menstrual period and three during the decidual period. There were seventeen with cystic glandular hyperplasia, one with adenocarcinoma, and one showing polyp. Lipoid was demonstrated in the glandular epithelium in 20 per cent of the cases, chiefly in the premenstrual and menstrual phases. Rich amounts of lipoid were found in the glandular lumen in 50 per cent of the cases during the stage of recovery. There was diffuse distribution of droplets of fat in the stroma, especially during the premenstrual phase. These observations agree with those of Ascheim, who found lipoids chiefly in the premenstrual phase.

JACOB KLEIN.

RELATION OF FATTY CHANGE OF THE AORTIC WALL TO ARTERIOSCLEROSIS. D. GÖRÖG, Virchows Arch. f. path. Anat. **287**:602, 1933.

Study of seventy-three aortas convinced Görög that the first anatomic lesion of aortic arteriosclerosis consists of a small, circumscribed area of fatty degeneration of the inner layer of the elastica. This finding seems to support the assumption that arteriosclerosis is due to an increased functional demand on the wall of the vessel. While the normal wall is capable of meeting the increased functional demand, this ability becomes impaired by persistently increased demand due to poisons, metabolic products or bacteria and to constitutional, congenital or familial factors.

W. SAPHIR.

MALFORMATION OF THE BASILAR BONE ASSOCIATED WITH OSSEOUS UNION OF THE ATLAS AND SKULL. P. SINZ, Virchows Arch. f. path. Anat. **287**:641, 1933.

A man, aged 27, died with symptoms of cerebellar tumor. Necropsy revealed a peculiar hypoplastic malformation of the basilar bone, with protrusion of the bone into the cranial cavity. The protrusion and the osseous union of the atlas



with the skull had led to displacement and narrowing of the foramen magnum, with consequent obstruction of the circulation of the cerebrospinal fluid and internal hydrocephalus. Sinz considers a primary disturbance in the development of the basilar bone the most likely cause of this unusual condition. W. SAPHIR.

INTESTINAL RHINOSCLEROMA. W. ROTTER and A. PEÑA CHAVARRÍA, *Virchows Arch. f. path. Anat.* **289**:345, 1933.

Involvement of the alimentary tract in rhinoscleroma has not been hitherto described. In a case of the disease which came to necropsy in Costa Rica the mucosa of the cecum and ascending colon from the ileocecal valve to the hepatic flexure was studded with nodular elevations. The nodules were composed of granulation tissue that contained Mikulicz' cells and was histologically identical with the tissue of the nasal and upper respiratory tract lesions of rhinoscleroma. From their experience with this disease the authors have found that the number of Mikulicz' cells may vary greatly. The cells are most numerous in the recent and most acute lesions. Sometimes the histologic diagnosis must be based on the presence of hyaline cells. When the histologic changes are not characteristic, the diagnosis must depend on the isolation of the specific encapsulated bacillus.

O. T. SCHULTZ.

PRIAPISM IN A CASE OF SEPSIS. S. SCHEIDEGGER, *Virchows Arch. f. path. Anat.* **289**:378, 1933.

A youth, aged 17, fell astride a timber on which he was working. He was able to continue his work after the accident. The next day he complained of severe sore throat. On the fifth day he was admitted to a hospital with a necrotizing tonsillitis, swelling and discoloration of the perineum and erection and edema of the penis. The priapism, which persisted after death, was due to thrombosis of the corpora cavernosa and of the pelvic veins. The priapism is ascribed to a combination of thrombosis due to the accident and sepsis due to the tonsillar infection.

O. T. SCHULTZ.

LYMPHOGRANULOMATOSIS OF THE VERTEBRAL COLUMN. E. WEGEMER, *Virchows Arch. f. path. Anat.* **289**:386, 1933.

A man, aged 31, died after a twelve year course of the disease. Although the bodies of the vertebrae had been greatly rarefied, their shape had not been much altered, because of the replacement of bone by dense fibrous tissue. The latter process was the result of fibrosclerosis of the lymphogranulomatous tissue that had invaded the vertebrae. The condition was not, as others have maintained, osteosclerosis but fibrosis of the specific granulation tissue. O. T. SCHULTZ.

CONGENITAL LOCALIZED OSTEOMYELITIS OF THE SKULL. W. LADEWIG, *Virchows Arch. f. path. Anat.* **289**:395, 1933.

A child born five weeks prematurely died nineteen hours after a normal delivery. At birth a tumor-like elevation of the scalp was noted in the left parietal region. Necropsy showed this to be an area of localized osteomyelitis of the parietal bone, with abscess. The inflammatory process had extended to the scalp and to the dura, where it was productive. There had been considerable new formation of bone. Diplococci could be seen in sections of the lesion. The process is held to have been the result of transplacental hematogenous infection. No history of infection of the mother during her pregnancy could be obtained.

O. T. SCHULTZ.

CHANGES IN THE HEART IN HYPERTHYROIDISM. A. DE CHÂTEL and W. MOLNÁR, Virchows Arch. f. path. Anat. **289**:557, 1933.

The hearts of seventeen patients who died of hyperthyroidism were subjected to histologic examination. The only changes noted were moderate hypertrophy, an increase in intracellular pigment, the presence of a few wandering cells with pigment and a diffuse slight increase in connective tissue. These changes were due to the increased functional activity of the cardiac muscle, followed by decreased activity and regression. Focal necrosis and inflammation, which has been described as toxic myocarditis due to hyperthyroidism, was not encountered.

O. T. SCHULTZ.

STUDY OF VASCULAR ANOMALIES BY MEANS OF INJECTION AND ROENTGENOGRAPHY. A. HINTZE, Virchows Arch. f. path. Anat. **289**:705, 1933.

Reproductions of seven excellent roentgenograms support Hintze's thesis of the value of contrast injection and roentgenography in the study of vascular anomalies. The method is the subject of a more or less general discussion. Three cases are described illustrating the differences between the vascular anomalies of congenital malformations and benign tumors, in which the variant vascular tree is looked on as physiologic and harmonic, and the abnormalities of the circulatory system in the presence of malignant tumors.

O. T. SCHULTZ.

SYMMETRICAL CORTICAL NECROSIS OF THE KIDNEYS. E. VON ZALKA, Virchows Arch. f. path. Anat. **290**:53, 1933.

Von Zalka found thirty-seven cases of symmetrical cortical necrosis of the kidneys recorded in the literature. Thirty-one of these were associated with pregnancy. He discusses the previous histologic findings in relation to two cases reported by himself, one in a girl, aged 13 years, and one in a man, 64 years old. In the child the renal involvement followed an attack of acute tonsillitis; in the man no history of antecedent illness could be obtained. Von Zalka agrees with the majority of other investigators that the necrosis is the result of thrombosis which occurs chiefly in the interlobular arteries and in the afferent and efferent arterioles of the glomeruli. He believes the thrombosis to be due to toxic injury of the endothelium.

O. T. SCHULTZ.

MASSIVE ANEMIC NECROSIS OF THE LUNG IN PNEUMONIA. M. REINISCH, Virchows Arch. f. path. Anat. **290**:75, 1933.

Reinisch reviews the small number of reported cases of pneumonia that terminated in massive anemic necrosis of the inflamed lung. The case which he reports was that of a 5 year old boy, who became suddenly ill the middle of January with a characteristic attack of pneumonia. The temperature returned to normal on the tenth day, but rose again two days later. Admission to the hospital occurred on February 12, at which time there was flatness on percussion of the lower part of the thorax on the left posteriorly; breath sounds were absent in this region. On the next day half a liter of purulent exudate was removed from the left side of the chest by thoracotomy. Death occurred on February 21. Necropsy revealed two relatively large, sharply defined areas of ischemic necrosis in the organizing upper lobe on the left. Reinisch divides the recorded cases of the condition into three groups. In the first two the essential factor is arterial thrombosis. The anemic character of the infarct is due to the inflamed state of the lung, which prevents the backflow of blood through the collateral capillaries. In the first group the lesion contains no bacteria, and the condition is termed aputrid anemic necrosis. In the second group bacteria are present; the condition is called septic anemic necrosis. In the third group, in which Reinisch places his own case, the essential factor is bacterial activity. Bacterial action causes thrombosis and necrosis; this group is called septic necrosis. The justification for making a distinction between the second and third groups does not appear well founded.

O. T. SCHULTZ.

LYMPHOGRANULOMATOSIS OF THE STOMACH AND XANTHOMATOSIS OF THE MESENTERY. W. BAUMGARTNER, *Virchows Arch. f. path. Anat.* **290**:97, 1933.

The reported case of Hodgkin's disease is one of the rare ones in which the lymphogranulomatous process is limited to the stomach. No other organs or lymph nodes were involved. In the wall of the jejunum and stomach and in the root of the mesentery were yellow nodules. These were composed of large foam cells filled with lipid. Baumgartner does not believe that the xanthomatosis was part of the lymphogranulomatous process.

O. T. SCHULTZ.

### Microbiology and Parasitology

RHEUMATIC MANIFESTATIONS IN SUBACUTE BACTERIAL ENDOCARDITIS IN CHILDREN. O. SAPHIR and S. A. WILE, *Am. Heart J.* **9**:29, 1933.

A study of ten cases of subacute bacterial endocarditis, eight in children and twelve in young adolescents who had been under observation since childhood, is reported. The histories revealed evidence of a preceding rheumatic infection in every instance. All hearts showed healed endocarditis, subacute bacterial endocarditis and Aschoff bodies in the myocardium, in addition to other changes. The blood revealed pure cultures of *Streptococcus viridans*, and smears from the vegetations showed gram-positive cocci in chains. The relation between the rheumatic infection and the final subacute endocarditis is discussed. A concurrence of these two conditions, though not definitely excluded, seems unlikely. Although allergy is an attractive explanation of rheumatic fever and its relation to subacute endocarditis, evidence is brought forward which seems to speak against rheumatic fever being an allergic phenomenon and the assumption that both conditions are manifestations of the same disease, differing only in the immunologic response of the host. At present the weight of evidence seems to be that the only relationship between rheumatic fever and subacute endocarditis is that the injury due to a previous rheumatic infection predisposes the valve to a subsequent endocarditis. The conclusion is reached that the Aschoff body is a characteristic structure and a specific reaction caused by the unknown virus of rheumatic fever. The circumscribed cellular infiltrations produced experimentally in hypersensitive animals, though morphologically resembling Aschoff bodies, are in no way characteristic of them. The use of strict criteria for the identification of Aschoff bodies is urged. The fact that in a number of cases the rheumatic manifestations gradually merged into the picture of subacute bacterial endocarditis may account for recent Aschoff bodies in the myocardium of patients who died of subacute bacterial endocarditis.

FROM THE AUTHORS' SUMMARY.

THE PERMEABILITY OF THE KIDNEY TO BACTERIA. M. H. BOOK, *Am. J. Path.* **9**:569, 1933.

*Staphylococcus aureus* injected into the circulation of the normal rabbit did not appear in the urine until between eight and twelve hours later. *Bacterium coli* was absent from the urine even up to forty-eight hours after injection. *Bacillus prodigiosus* failed to reach the urine up to twenty-four hours after injection. The examination of ordinary paraffin sections to determine the exact site of a few individual bacteria is not entirely reliable. The method of incubating the kidney before fixation and staining not only facilitated greatly the finding of the bacteria, but permitted a more dependable analysis of the location of the organisms from which the colonies had developed. By means of such examination it was seen that the bacteria never reached the capsular space about the glomeruli or the lumens of the tubules in the first few hours after injection and hence were not excreted. The probability is that they did not get beyond the vascular system until they had damaged the walls of the latter.

AUTHOR'S SUMMARY AND CONCLUSIONS.

ACTINOBACILLOSIS OF MAN. D. C. BEAVER and L. THOMPSON, *Am. J. Path.* 9:603, 1933.

Three cases of actinobacillosis in man are now well authenticated. The case concerned here offered the first opportunity for pathologic study of the lesions in a human being. The lesions were essentially similar to those that have been described in cattle except that they were much more widespread, and sulphur granules such as occur in the bovine lesions apparently did not occur. The lesions may be described as granulomatous abscesses severely affecting the lungs, liver and spleen. Lesions similar to those observed in man and cattle were produced in animals experimentally. Glanders and tularemia were considered in the differential diagnosis, but both seemed to be definitely ruled out by the bacteriologic and serologic investigations. The organism isolated reveals a close cultural and antigenic relation to *Actinobacillus Lignièresi*, and a more distant relation to *Pfeifferella mallei* and *Bacillus Whitmori*. Thompson has previously shown that *A. Lignièresi* of bovine and human origin, *Pf. mallei* and *B. Whitmori* are antigenically interrelated and has proposed that they be included in a common genus. This relationship, as far as the organism in the present case is concerned, is also confirmed from the pathologic point of view, for the lesions in man and the experimental lesions in animals were similar to those of glanders and also exhibited similarities to the lesions of bovine actinobacillosis. Although the organism under consideration revealed minor cultural differences and antigenic phenomena somewhat different from those of a typical strain of *A. Lignièresi* of bovine origin, nevertheless it seems justifiable to regard the organism as a variant of the usual bovine strain of *A. Lignièresi*.

AUTHORS' SUMMARY.

THE PERSISTENCE OF TUBERCULOUS INFECTIONS. H. E. ROBERTSON, *Am. J. Path.* 9:711, 1933.

Tuberculous infections may occur and pursue their entire course without demonstrable clinical phenomena, that is, without attracting the attention of the patient or a physician to their presence. Recognized tuberculous infections may subside and be regarded throughout remaining life as healed and still remain continuously active. Apparently healed tuberculous lesions may become clinically active after varying intervals. No form of physical examination can assure any person that he or she does not harbor the menace of active tuberculous infection. The safest rule for physicians and patients alike is to regard tuberculosis as possessing an ever present potentiality for becoming active. One can almost say: "Once infected, always infected."

AUTHOR'S CONCLUSIONS.

TUBERCLE BACILLI IN THE BLOOD IN ADVANCED PULMONARY TUBERCULOSIS. H. J. CORPER and A. P. DAMEROW, *Am. Rev. Tuberc.* 28:118, 1933.

Tubercle bacilli were not found in the blood of 120 patients with advanced and generalized tuberculosis. Each blood specimen was submitted to four methods of examination for tubercle bacilli, namely, inoculation in guinea-pigs and three different cultural methods. While control tests proved the efficacy of the cultural methods used for discerning small numbers of tubercle bacilli in human and animal blood, acetic acid, as recommended by Loewenstein, destroyed small numbers of tubercle bacilli while permitting saprophytic acid-fast bacilli to survive, thus vitiating the results obtained with this reagent. Over 200 smaller specimens obtained as a routine from patients in a sanatorium and examined by two cultural methods, the tissue substrate method and the sulphuric acid-crystal violet-potato-cylinder method, did not yield a single culture positive for tubercle bacilli. Of the 120 patients whose blood was examined, 2 yielded saprophytic acid-fast bacilli which were proved not to be pathogenic tubercle bacilli. Tubercle bacilli, when present in small numbers, grow well on a sulphuric acid-treated and sodium bicarbonate-neutralized or sodium hydroxide-neutralized blood residue or on inspissated defibrinated blood, making it appear that acid-treated or heat-treated

or sterilized blood pigments are not detrimental to the growth of tubercle bacilli. The macroscopic type of culture obtained on the blood medium differs from that seen on potato or that on egg, which also differ from each other. The congo red-potato flour-egg medium (Loewenstein) is a good one for human and bovine tubercle bacilli, but it is not better than a simple potato cylinder or inspissated egg-yolk medium. It appears that human or bovine tubercle bacillæ, in the sense that tubercle bacilli circulate in the blood for a fairly long time or that the bacilli multiply in the blood, are not borne out by these studies, although it is not intended to convey the impression that occasional embolic disseminations (showers) from disintegrating tuberculous foci do not occur or that there are not terminal periods in the course of the disease when showers of caseous products containing visible bacilli may make it possible to find them in the blood of man and experimental animals. Such a condition of tubercle bacilli in the blood is far from being the common event. Embolic showers, when they occur in man, are rapidly removed from the circulation, in the usual case of tuberculosis.

H. J. CORPER.

THE COCKROACH AS A POSSIBLE CARRIER OF TUBERCULOSIS. HENRY C. READ, Jr., *Am. Rev. Tuberc.* **28**:267, 1933.

The cockroach may be considered a possible mechanical carrier of tuberculosis for the following reasons: 1. All smears of the intestinal tracts of cockroaches allowed to feed on infected sputum showed tubercle bacilli. 2. The organisms recovered from the intestinal tract were viable, for they produced lesions in guinea-pigs. 3. Microscopic sections did not show the bacilli to be present in the tissues, thus showing that they remain in the intestinal tract.

H. J. CORPER.

TUBERCULOSIS OF HUMAN ORIGIN IN AN AMAZON PARROT. W. R. HINSHAW, *Am. Rev. Tuberc.* **28**:273, 1933.

A case of tuberculosis in a male "Amazon parrot" (genus *Amazona*) is reported. There was a history of the parrot's being the pet of a patient with tuberculosis, and inoculations in animals indicated that the bird was suffering from the human type of the disease.

H. J. CORPER.

POLIOMYELITIS VIRUS. J. R. PAUL and J. D. TRASK, *J. Exper. Med.* **58**:513, 1933.

That qualitative differences exist between so-called human and passage strains of poliomyelitic virus has been established by the following observations: (a) The experimental disease induced by two human strains usually failed to protect monkeys against a subsequent infection by a passage strain, and in the few instances in which the reverse experiment could be made a similar lack of protection was observed. (b) In some human serums the neutralizing power for a human strain differed qualitatively from that for a passage strain. The length of time between the intracerebral inoculations of heterologous strains has been found to be an important factor bearing on the results of the reinoculation experiments reported. Within the intervals used, the greater the period between the original infection and the reinoculation with a heterologous strain the less was the degree of cross-immunity observed.

AUTHORS' SUMMARY.

THE CULTIVATION OF VACCINE VIRUS FOR JENNERIAN PROPHYLAXIS IN MAN. T. M. RIVERS and S. M. WARD, *J. Exper. Med.* **58**:635, 1933.

A dermal strain of vaccine virus has been subjected to 99 successive cultural passages. This procedure led to a diminution in the pathogenicity of the active agent for the rabbit. By repeated testicular passages in rabbits, however, the virus regained its pathogenicity for that host. New cultures were initiated with

the revived virus. A culture strain of virus that has been twice revived in this manner has remained fairly stable for the rabbit through 60 cultural passages, and it produces mild, yet effective vaccinal reactions in man. Virus in early cultures was not attenuated for man, but later cultures of the original strain and cultures of the second and third revived strains produced mild reactions without fever and discomfort to the patients. Intradermal vaccinations with the culture virus are safe and satisfactory. With the culture virus 118 infants and children have been inoculated, and in 100 of them a positive reaction occurred. The culture virus produced a refractory state to a standard dermal strain of calf lymph and vice versa. Culture virus stored in 50 per cent neutral glycerin at  $-10^{\circ}\text{C}$ . or at  $+3^{\circ}\text{C}$ . maintained a considerable amount of its activity for at least one year. Desiccated culture virus sealed in tubes maintained some of its activity when stored at  $37^{\circ}\text{C}$ . for five weeks. Fresh cultures can be initiated without difficulty from desiccated virus or from virus that has been stored with or without glycerol.

AUTHORS' SUMMARY.

CULTIVATION OF PSEUDORABIES VIRUS. E. TRAUB, J. Exper. Med. **58**:663, 1933.

Pseudorabies virus has been cultivated in series in rabbit testicle, guinea-pig testicle and chick embryo medium, and its growth requirements have been studied. Intranuclear inclusions similar to those produced by pseudorabies virus in vivo have been found in cultures on rabbit testicle medium. The virus has not changed its pathogenic properties for rabbits, guinea-pigs or mice during the course of cultivation.

AUTHOR'S SUMMARY.

THE FORMATION OF GREEN PIGMENT FROM HAEMOGLOBIN BY THE PNEUMOCOCCUS. P. D. HART and A. B. ANDERSON, J. Path. & Bact. **37**:91, 1933.

An olive green precipitate was formed when small quantities of laked blood were incubated aerobically with broth cultures of the pneumococcus to which alkaline phosphate solutions had been added. This precipitate could be separated, washed and extracted with dilute alkali to give a green solution. Suitable conditions for the formation of the pigment, including the optimal range of  $p_{\text{H}}$ , are described. When the mixtures were incubated anaerobically, reduced hemoglobin was formed; on subsequent aerobic incubation, this was converted into the green pigment. Aerobic or anaerobic cultures allowed to autolyze in air with phosphate solutions rapidly lost their ability to produce green pigment from blood. Cultures autolyzed under anaerobic conditions remained active for longer periods. Sterile filtrates of unautolyzed anaerobic cultures were found to be inactive. Active filtrates were obtained from anaerobic cultures only after disintegration of the organisms. The activity of sterile filtrates was rapidly destroyed by aeration, by the addition of hydrogen dioxide or by heating to  $55^{\circ}\text{C}$ . Addition of a solution containing catalase to cultures or filtrates retarded the destruction of activity brought about by exposure to air. Suspensions of washed organisms in phosphate buffer were themselves unable to produce the green pigment from blood but could be activated by meat extract and other substances. Extracts prepared from such washed organisms were not activated in this manner. Crystalline oxyhemoglobin, methemoglobin and, to a less extent, carboxyhemoglobin could replace laked blood as a source of the green pigment. The green pigment has not yet been identified; its physical and chemical properties do not correspond with those of any of the common derivatives of hemoglobin. The nature of the enzyme system involved in the formation of the green pigment and its possible identity with the system described by Neill and Avery (1924) are discussed.

AUTHORS' SUMMARY.

A VIRUS DISEASE OF THE CANARY OF THE FOWL-POX GROUP. F. M. BURNET, J. Path. & Bact. **37**:107, 1933.

The disease of canaries described by Kikuth and Gollub is caused by a virus closely resembling certain strains of the virus of fowl pox. The main histologic features of the disease are: (a) a proliferation of the dermal epithelium with cyto-

plasmic inclusions; (b) an inflammatory response of predominantly mononuclear cells showing characteristic vacuolation of the cytoplasm, and (c) a massive accumulation in the infected lung of large mononuclear cells containing specific cytoplasmic inclusions.

The virus produces massive lesions when it is inoculated into the chorio-allantoic membrane of the developing egg. An improved technic for this type of inoculation is described. By filtration and photomicrographic methods the diameter of the particles of the virus is estimated to be approximately 0.16 micron. The disease is uniformly fatal to canaries, and no success has been attained in attempts to immunize them with killed virus. The virus is pathogenic for sparrows, producing typical lesions. Only an insignificant lesion is produced in the fowl, but a more definite transmissible inflammatory condition is produced in the pigeon. In neither of these species are epithelial inclusions found. Of three strains of the virus of fowl pox tested, one ("Dalling") produced lesions in the canary similar to those produced by Kikuth's virus. The other two strains and a strain of the virus of pigeon pox have failed to induce specific lesions.

AUTHOR'S SUMMARY.

CELLULAR RESPONSE OF VITREOUS HUMOUR TO BACTERIA, BLOOD AND VITAL DYES. W. A. GRAY, *J. Path. & Bact.* **37**:137, 1933.

The first cells to respond to the presence of irritation in the vitreous are polymorphonuclear leukocytes. They are quickly followed by macrophages; the macrophages increase, in the later stages of the inflammation, as the polymorphonuclear leukocytes diminish. The cellular exudate is derived almost entirely from adjacent vascular structures, but occasionally there is a proliferation of periadventitial histiocytes at the optic disk. The area of the wound produced during the injection of the irritant is the first and for a time the principal source of supply of the emigrating cells, but sooner or later they are derived from the optic disk and the pars plana of the ciliary body. Micro-organisms introduced into the vitreous tend to spread rapidly to the anterior chamber, to the ciliary body, especially the pars plana, and to the optic disk, where they call forth an inflammatory reaction.

AUTHOR'S SUMMARY.

CLASSIFICATION OF DYSENTERY-COLI BACTERIOPHAGES. F. M. BURNET, *J. Path. & Bact.* **37**:179, 1933.

There are sharp distinctions among bacteriophages in regard to rate of photodynamic inactivation by methylthionine chloride, U. S. P. (methylene blue), ability to lyse in the presence of citrate and rate of inactivation by strong urea solutions. Phages falling in a single serologic group as previously defined are approximately uniform in their response to any of these tests. Strong urea solutions inactivate large particle phages with great rapidity and (with one exception) are much less active on small particle phages. Several acridine dyes can be used for photodynamic inactivation of suitable phages.

AUTHOR'S SUMMARY.

THE STABILITY OF THE MITIS, INTERMEDIATE AND GRAVIS TYPES OF *B. DIPHTHERIAE*. M. H. CHRISTISON, *J. Path. & Bact.* **37**:243, 1933.

The three main types of *Bacillus diphtheriae* undergo marked variation in structure of colony in vitro, particularly after growth in bouillon. Rough variants derived from the mitis strain would at sight be classified as gravis and derivatives of gravis colonies as mitis. Variation also occurs in the type of growth in bouillon and in the production of hemolysin. Changes in structure of colony are observed not only in strains obtained from Leeds, but also in those isolated in Edinburgh. These findings and the occasional isolation of "atypical" strains suggest that dissociation occurs in vivo as well as in vitro. Gravis strains ferment starch irrespective of the structure of the colony, whereas intermediate and mitis forms and their

variants fail to do so. It appears, therefore, that in identifying the gravis type the fermentation of starch is a more reliable criterion than the structure of the colony.

AUTHOR'S SUMMARY.

MECHANISM OF ANTHRAX INFECTION. A. BOQUET and A. SAENZ, *Ann. Inst. Pasteur* 50:311, 1933.

Hemorrhagic or necrotic exudate was considered less significant than progressive bacteremia. Boquet and Saenz take exception to Besredka's statement that the skin exclusively is sensitive to anthrax organisms. Presumably owing to the greater activity of the wandering cells and histiocytes in the subcutaneous tissue the latter was demonstrated to be four or five times less receptive than the skin. Experiments with inhalation resulted in a fatal septicemia emanating from the pulmonary alveoli, as appears to occur in human infection. Intraperitoneal injections often resulted in aborted infections, but when infection occurred it appeared to be due to multiplication of the organisms in the liver and spleen rather than to accidental contamination of the skin. Organisms fed in massive doses were at times noted in the blood stream from two to eight hours later, but no infection resulted, owing to rapid phagocytosis in the liver, spleen and bone marrow.

FROM THE AUTHORS' CONCLUSIONS.

FILTRABLE ELEMENTS IN TUBERCULOSIS. M. C. NINNI, *Ann. Inst. Pasteur* 50:504, 1933.

Ninni believes that the invisible filtrable granular elements secured from young cultures of the tubercle bacillus and from infected organs are living and are capable of multiplication in vivo and in vitro; that the lymphatic Calmette-Valtis type of tuberculosis, without tubercles, is characteristic of their presence; that they may give rise to typical organisms, but are not to be confused with variously suggested artefacts; that young cultures of these forms are toxic for nerve cells; that they may pass the intact placenta of pregnant females, possibly causing death of the fetus or progressive malnutrition of the new-born, or producing immunity, and that the ultravirus together with the Koch bacilli constitute the virus of tuberculosis, thus affording, according to the balance, the great variety of clinical forms encountered.

FROM THE AUTHOR'S CONCLUSIONS.

HUMAN TUBERCLE BACILLUS ON BILE MEDIUM FOR TWENTY-TWO YEARS. A. CALMETTE, C. GUÉRIN and L. NÈGRE, *Ann. Inst. Pasteur* 50:599, 1933.

The original BCG strain of the tubercle bacillus was of bovine origin. A strain of human derivation and of medium virulence was secured in 1908 from Trudeau's laboratory at Saranac, N. Y. Beginning March 10, 1910, a series of 418 successive parallel transfers were made in an alkaline medium containing potato, ox bile and glycerin. The virulence for guinea-pigs and rabbits appeared to increase in both the human and the bovine strain at first, but weakened after the thirty-fifth transfer. By 1921 a dose equivalent to 1 mg. failed to affect these animals. The avirulence and the antigenic and immunizing properties of the human strain appeared to be equivalent to those of BCG. No substitutions in BCG vaccine were contemplated, but the stability of the parallel strain in a study of this type was considered significant.

M. S. MARSHALL.

PATHOGENESIS OF MENINGOCOCCIC MENINGITIS. P. ZDRODOWSKI, *Ann. Inst. Pasteur* 50:651, 1933.

A fatal meningitis was produced in rabbits by a subarachnoid injection of two billion freshly isolated meningococci. The virulence was quickly lost on serum medium, but was retained for several months when the organisms were transferred



every two weeks on Dorset's medium. Very satisfactory viability (from two to three months) and virulence (from one to one and a half months) were maintained when a liquid egg medium under paraffin oil was used. Partially attenuated strains were reactivated by passage through animals, using either augmented doses or homologous endotoxin prepared by heating an emulsion at 100 C. The minimum lethal dose for a rabbit weighing 1.5 Kg. appeared to be between one and two billion organisms. The apparent virulence was dependent on the combined infectiousness of living organisms and the pathogenicity of the toxic substance, which accounted for the relatively large dose needed.

FROM THE AUTHORS' CONCLUSIONS.

REPEATED INJECTIONS OF BCG IN MONKEYS. I. M. LÉVITAN and D. D. LOKHOFF, *Ann. Inst. Pasteur* **50**:749, 1933.

Repeated vaccinations of monkeys with BCG were absolutely innocuous. They conferred a clearly increased resistance to subsequent virulent infections. Neither repeated vaccinations or spontaneous disease nor poor nutrition increased the virulence of BCG. The use of BCG on tuberculous monkeys exercised no noticeable effect on the evolution of virulent tuberculous processes.

AUTHORS' CONCLUSIONS.

AUTOCHTHONOUS RABIES IN AFRICA. S. NICOLAU, C. MATHIS and V. CONSTANTINESCO, *Ann. Inst. Pasteur* **50**:778, 1933.

There exists in the western part of the French mandate in Africa a special disease of mad dogs, in the local dialect "Oulou-fato." Several strains of the fixed virus were established in rabbits. The Negri bodies were most apparent in the basal optic nerve. The virus was invisible and was not cultured successfully. It was effective in dilutions of 1:500 (street virus) and 1:20,000 (fixed virus). Glycerin attenuated the street virus in three or four weeks, and the fixed virus, in three or four months. The substances associated with the virus were electro-negative. Rabbits and guinea-pigs were more susceptible than rats, mice, dogs or chickens. Subdural inoculation with the street virus induced, in rabbits, a rabies the paralytic phase of which was preceded by a stage of excitement and intense contraction. The fixed virus induced a severe paralytic reaction of short duration. Rabbits were killed by the latter when it was injected by skin, muscle, eye or vein or in the region of the sciatic nerve. The neurotropic properties were marked. The histologic changes in the central, visceral and peripheral nervous systems were those of rabies. The Negri bodies differed in their staining properties, but were almost always blue by Mann's method, without the oxyphilous character of the cytoplasmic granules found in rabies. The pleomorphism was accentuated. Neurons in advanced stages of degeneration often had inclusions. Cross immunity for classic rabies was perfect. The extensive experimental report is accompanied by a two page color plate.

FROM THE AUTHORS' CONCLUSIONS.

TYPHUS IN RATS. CHARLES NICOLLE, *Arch. Inst. Pasteur de Tunis* **21**:349, 1933.

Transfer experiments with the historic strain of typhus (Tunis) in rats indicated that a silent infection might be induced by inoculation of brain material into the peritoneal cavity. A definite number of transfers, twelve or thirteen, appeared to be the limit. Transfer was effected from animals killed from the seventh to the sixteenth day after inoculation, and from four days before to two days after the appearance of fever in control guinea-pigs. Failure to propagate the virus over a longer period was attributed to a failure of the virus to adapt itself to an unnatural host.

FROM THE AUTHOR'S CONCLUSIONS.

SURVIVAL OF TYPHUS VIRUS IN BRAIN TISSUE. CHARLES NICOLLE and J. LAIGRET, Arch. Inst. Pasteur de Tunis **21**:357, 1933.

Virulent Toulon virus was present in the brains of rats for as long as sixty-eight days. The virus survived in the brains of guinea-pigs for not longer than thirty-three days. Several strains of Mexican virus were not demonstrated after fifty days in rats or thirty-four days in guinea-pigs. With the Tunis virus the situation was reversed; rats were free from the virus twenty days after inoculation, and guinea-pigs irregularly showed its presence at this time. Brains of rats given the virus of Rocky Mountain spotted fever were free from it on the twentieth day and guinea-pigs on the twenty-eighth day, but the end-point was not determined.

M. S. MARSHALL.

### Immunology

TITRATION OF YELLOW FEVER VIRUS IN STEGOMYIA MOSQUITOES. N. C. DAVIS, M. FROBISHER, JR., and W. LLOYD, J. Exper. Med. **58**:211, 1933.

Titration was made of the virus of yellow fever in mosquitoes of the genus *Stegomyia*, rhesus monkeys being used as test animals. Immediately after becoming engorged with highly infectious blood, the average mosquito contained between 1,000,000 and 2,000,000 lethal doses of virus. The titer of freshly ingested blood was as high as 1,000,000,000 lethal doses of virus per cubic centimeter. During the fortnight succeeding the ingestion of infectious blood there occurred a reduction of titratable virus to not more than 1 per cent of that present in the freshly fed insects. The titer was somewhat higher at later periods. This rise in titer signified possibly, not a multiplication, but merely an increase of extracellular virus and of that easily freed by grinding to a titratable form. At no later stage did the quantity of titratable virus equal that demonstrable in freshly fed insects.

AUTHORS' SUMMARY.

PRECIPITIN REACTION IN YELLOW FEVER. T. P. HUGHES, J. Immunol. **25**:275, 1933.

Serums taken from monkeys shortly after recovery from severe yellow fever infections possess a precipitin capable of reacting with a precipitinogen which occurs in the blood of monkeys during the period of acute illness. This precipitinogen is not the virus of yellow fever, but appears to be associated with a protein of the albumin fraction. Its concentration reflects the severity of the illness. It disappears with recovery, after stimulating the formation of a precipitating antibody. This resulting precipitin is entirely independent of the protective antibody resulting from an infection. A similar precipitin occurs in the serum of patients shortly after recovery from a severe yellow fever infection. This precipitin reacts with the precipitinogen occurring in the blood of monkeys during the acute phase of the illness.

AUTHOR'S SUMMARY.

THE VOLUME OF PRECIPITATE IN PRECIPITIN REACTIONS. F. S. JONES and R. B. LITTLE, J. Immunol. **25**:381, 1933.

A procedure has been described whereby the quantity of precipitate resulting from the action of precipitin and antigen can be measured. At one point in titrations, as usually practiced, the greatest quantity of precipitate is formed, and we have called this point the zone of maximum precipitation. With this as a basis we have been able to show that the quantity of precipitate varies proportionately with changes in the quantity of either antigen or immune serum. A linear type of reaction has been demonstrated. In addition, similar linear relationships have been shown in the determinations of antigen and antibody in the supernatant fluid after precipitation has taken place. We have not been able to show that in a given series of cow serum and its precipitin either the antigen or the antibody is completely utilized. When a single antigen, such as egg albumin, was employed with

its precipitin there occurred within a narrow zone complete binding of both the antigen and the antibody, while in either direction one or the other was still present in active form.

AUTHORS' SUMMARY.

EFFECT OF DISSOCIATION ON ANTIGENIC BEHAVIOR OF *SALMONELLA* AND *SHIGELLA* CULTURES. G. M. MACKENZIE and HELEN FITZGERALD, J. Immunol. **25**:397, 1933.

The antigenic components of *Salmonella* and *Shigella* may show qualitative and quantitative changes due to dissociation. Components not demonstrable in the original culture may appear in the variants. The more marked the change in the colony form, the more marked are the alterations in serologic behavior. Changes in antigenic behavior due to dissociation are qualitatively different from those caused by heat.

EDNA DELVES.

CULTURES AND BROTH FILTRATES OF *STAPHYLOCOCCI*. E. L. BURKY, J. Immunol. **25**:419, 1933.

Intracutaneous or intravenous injection of staphylococcus toxin into rabbits is followed by the development of an active immunity to the toxin and by the appearance of precipitating substances in the serum. The intracutaneous method is as effective as the intravenous, and the rate of mortality is less. In certain rabbits antibodies capable of passively protecting rabbits against multiple lethal doses of toxin are developed, independently of precipitins. Certain serums, supposedly immune and with high precipitin titers, seem to increase the lethal action of the toxin. The serums of rabbits immune to toxin precipitate only with the filtrates of strains of staphylococci pathogenic for rabbits. Strains of staphylococci pathogenic for rabbits have been recovered only from definite lesions in man and rabbits. This relation suggests a method for the classification of staphylococci and a diagnostic aid in cases in which staphylococci are isolated and in which the etiologic significance is doubtful.

AUTHOR'S SUMMARY.

SEASONAL CHANGES IN THE CATAPHORETIC VELOCITY AND VIRULENCE OF *STREPTOCOCCI*. E. C. ROSENOW, J. Infect. Dis. **53**:1, 1933.

A method yielding reliable results in the isolation of streptococci and the study of their cataphoretic velocities is described. Streptococci as isolated from atria of infection, especially from the nasopharynxes of well persons and persons ill with various nonepidemic or epidemic diseases, have characteristic cataphoretic velocities and sometimes also demonstrably characteristic degrees of virulence. A striking parallelism was found between the cataphoretic velocity of streptococci isolated from the nasopharynxes of persons ill with epidemic diseases, such as the common cold, influenza and poliomyelitis, and that of streptococci isolated at the same time from the raw milk supply, as well as between the velocities of both in their return to normal after the epidemics disappeared. The velocity of streptococci isolated from the nasopharynxes of normal persons and that of streptococci isolated from the nasopharynxes and other atria of infection, including the apexes of pulpless teeth, of persons suffering from chronic diseases such as encephalitis and arthritis shift toward that of the streptococci prevalent in the throats of persons suffering from the epidemic respiratory diseases in question, especially the common cold and influenza, and return, respectively, to the normal and to that characteristic of the chronic disease after the epidemics subside. Despite these striking revelations with respect to the etiologic importance of streptococci it is not concluded that the "virus" of the common cold or other organisms also isolated sometimes in this condition and influenza have no etiologic significance in these diseases. The increase in incidence of acute systemic diseases, such as arthritis, the exacerbations of symptoms in chronic infective diseases and the rise and fall of epidemic waves of infectious diseases according to season, in the light of these results, become more explicable.

AUTHOR'S SUMMARY.

MUCOID ENCAPSULATED STREPTOCOCCI IN SCARLET FEVER. I. PILOT and D. J. DAVIS, *J. Infect. Dis.* **53**:29, 1933.

Encapsulated hemolytic streptococci forming mucoid colonies on ascites blood agar were isolated in sporadic cases of scarlet fever. A strain isolated from bovine mastitis causing milk-borne epidemic scarlet fever also yielded such colonies and capsules. Certain differences are described between these streptococci of scarlet fever and *Streptococcus epidemicus* of septic sore throat. Toxins of these streptococci from epidemic and sporadic scarlet fever correspond to the specific toxin of the streptococcus of scarlet fever and not to the toxin of *Str. epidemicus* of septic sore throat. The encapsulated streptococci of septic sore throat and scarlet fever may be similar in their behavior as the cause of milk-borne infections, both producing bovine mastitis from which an outbreak of septic sore throat or of scarlet fever may develop. That such a similarity exists may be further determined by proper studies of milk-borne scarlet fever. The problem of the control of milk-borne scarlet fever then will become identical with that of the control of septic sore throat.

AUTHORS' SUMMARY.

MICRO-ORGANISMS WHICH DECOMPOSE THE SPECIFIC CARBOHYDRATE OF PNEUMOCOCCUS, TYPES II AND III. G. M. SICKLES and M. SHAW, *J. Infect. Dis.* **53**:38, 1933.

A description is given of several strains of soil organisms isolated from decaying organic matter in different localities which are able to decompose the specific polysaccharide of *Pneumococcus*, type III. One of the strains, unlike that previously described by Avery and Dubos, also utilizes agar. Experiments with the protective and curative effect, in mice, of the enzyme obtained from these strains confirmed, in general, the results of Avery and Dubos. A soil organism which decomposes the specific polysaccharide of *Pneumococcus*, type II, and which differs markedly from the strains that decompose the carbohydrate of *Pneumococcus*, type III, is also described. From this organism no soluble enzyme was obtained. Neither the specific "cellular carbohydrate" of type II nor that of type III (Wadsworth and Brown) produced purpura in mice after it had been acted on by the homologous decomposing organism.

AUTHORS' SUMMARY.

COMPLEMENT FIXATION IN VACCINIA AND IN VARIOLA. R. F. PARKER and R. S. MUCKENFUSS, *J. Infect. Dis.* **53**:44, 1933.

By the technic of complement fixation a specific reaction between the virus of vaccinia and the immune serum has been demonstrated. The reaction is influenced by the potency of the serum and by the time allowed for fixation, and apparently many of the failures of other workers to demonstrate it have been due to the use of serum of insufficient activity or to too short a period of fixation. Active virus in the antigen is not necessary for fixation; preparations which had been boiled or passed through a Berkefeld N candle and in which no virus could be demonstrated still fixed complement with immune serum, although not in as great a dilution as before treatment. These results are probably comparable with those obtained by Cragie in his study of the antigens taking part in the reaction of flocculation. Specific complement fixation in smallpox has also been demonstrated by using a specific antivaccinal rabbit serum and the fluid from the pustules of the disease. This has been made possible by an adaptation of the test to a small volume, the reagents being measured in drops. Various materials from persons with different cutaneous diseases and from the vesicles of varicella have been tested, without false positive results. With vaccinia and variola the results have been uniformly positive when the material was collected before the thirteenth day of the eruption. Difficulty due to bacterial contamination has been avoided by the use of serum prepared against a bacteria-free antigen. Serums from ten patients with variola were tested, with

partial or complete fixation in three. Complement fixation with a specific anti-vaccinal serum may be of assistance in the early diagnosis of smallpox.

AUTHORS' SUMMARY.

**INFLUENCE OF HIGH FREQUENCY DISPLACEMENT CURRENTS ON BACTERIA.**

F. W. FABIAN and H. T. GRAHAM, *J. Infect. Dis.* **53**:76, 1933.

In a high frequency displacement current of 10 megacycles and an intensity of 0.08 ampere colon bacilli increased in number nearly 300 per cent during a period of three hours. When the intensity of the displacement current was increased approximately ten times the lethal effect of the current became evident. Of the three frequencies studied, 7.5, 10 and 15 megacycles, 10 megacycles was the most effective, 7.5 megacycles was the least effective, and 15 megacycles occupied an intermediate position with respect to killing action on bacteria. The high frequency displacement currents produced a regular order of death in bacteria. When the logarithm of the number of bacteria surviving at regular intervals was plotted against time a typical survivor curve resulted.

AUTHORS' SUMMARY.

**DISSOCIATION OF CLOSTRIDIUM WELCHII.** H. R. LIVESAY, *J. Infect. Dis.* **53**:125, 1933.

After prolonged cultivation on the surface of suitable artificial mediums, *Clostridium Welchii* produced rough colonies made up of encapsulated rods arranged in chains or filaments. From such variants there sometimes developed pigmented subsurface rootlets growing into the underlying medium. *Cl. Welchii* planted deep in shake tubes produced variants that formed colonies morphologically similar to the characteristic colony of *Clostridium sporogenes*. However, owing to the fact that it was impossible to isolate *Cl. sporogenes* from these colonies and to the fact that in every instance the organisms present gave the characteristic reaction of *Cl. Welchii*, it is believed that they were variants of the latter organism. Under certain circumstances *Cl. Welchii* growing on the surface of solid mediums produced pigmented subsurface rootlets or colonies; in shake cultures, this clostridium sometimes grew as ball-shaped colonies. The results indicate that pigmentation and subsurface penetration do not always mean contamination, but may result from dissociation of *Cl. Welchii*.

AUTHOR'S SUMMARY.

**THE SHWARTZMAN PHENOMENON.** A. M. PABST and S. E. BRANHAM, *Pub. Health Rep.* **48**:639, 1933.

Serum neutralization of the Shwartzman phenomenon produced by filtered meningococcic washings is not restricted to antimeningococcic serums, but also occurs with antipneumococcic, antidyenteric and antigonococcic serums and with diphtheria antitoxin, as well as with normal horse and rabbit serums. This non-specific neutralization is so frequent and so marked that it seems to limit the usefulness of the Shwartzman phenomenon in the evaluation of therapeutic antimeningococcic serums.

AUTHORS' SUMMARY.

**IMMUNOLOGICAL STUDIES WITH PHAGE-COATED BACTERIA.** F. M. BURNET, *Brit. J. Exper. Path.* **14**:93, 1933.

Bacteria on which certain bacteriophages have been adsorbed can be specifically agglutinated by antibacteriophage serum and can be used to absorb antibacteriophage from an immune serum. Cross-absorption experiments by this technic have been carried out with two antigenically related bacteriophages and their antisera. With one bacteriophage it has been shown that bacteriophage inactivated by a solution of formaldehyde can be adsorbed by bacteria and endow them with the specific reactivity toward the corresponding antibacteriophage.

AUTHOR'S SUMMARY.

A SPECIFIC SOLUBLE SUBSTANCE FROM BACTERIOPHAGES. F. M. BURNET, Brit. J. Exper. Path. **14**:100, 1933.

A bacteriophage-specific soluble substance is present in bacteriophage-free ultrafiltrates of lysed cultures; it can be demonstrated by its blocking effect on the specific bacteriophage-antibacteriophage reaction. The specific soluble substance is not adsorbed by bacteria sensitive to the corresponding bacteriophage and does not represent a soluble lysin. The activity of the specific soluble substances is reduced by heating to temperatures above 65 C., and is completely lost after heating for thirty minutes at from 90 to 93 C. By the addition of relatively large amounts of the specific soluble substances to bacteriophage-antibacteriophage mixtures the process of neutralization can be stopped and to some degree reversed. Rabbits inoculated with ultrafiltrates containing the specific soluble substances show a slight appearance of bacteriophage-inactivating antibodies.

AUTHOR'S SUMMARY.

IMMUNOLOGICAL STUDIES WITH THE VIRUS OF PSITTACOSIS. S. P. BEDSON, Brit. J. Exper. Path. **14**:162, 1933.

The serums of persons convalescent from psittacosis possess no demonstrable neutralizing power, but will fix complement in the presence of a psittacosis antigen. Similarly, the serums of hyperimmunized guinea-pigs contain considerable antibody strength demonstrable by complement-fixation or agglutination tests, but only a low neutralizing power. The value of the complement-fixation reaction in the diagnosis of psittacosis is discussed. Virus which has been rendered inactive by means of a solution of formaldehyde can produce a considerable degree of immunity in mice. Virus to which a solution of formaldehyde has been added can be steamed for twenty minutes without much loss of immunizing power, but steaming alone has a deleterious effect.

AUTHOR'S SUMMARY.

HAY FEVER REAGIN-ALLERGEN. D. HARLEY, Brit. J. Exper. Path. **14**:171, 1933.

Mixtures of reagin and allergen produce reactions in normal skins. Reagin is not inactivated by allergen in vitro. Allergen is bound by reagin in vitro. Allergen bound by reagin can be freed by heating to destroy the reagin.

AUTHOR'S SUMMARY.

THE TOXIC EFFECTS OF STAPHYLOCOCCAL FILTRATES INTRODUCED ENTERALLY. GRIZEL R. BORTHWICK, Brit. J. Exper. Path. **14**:236, 1933.

When staphylococcus toxin is added to gastric contents in vitro a slightly acid ( $p_H$  6.8) or a slightly alkaline ( $p_H$  7.8) reaction impairs its activity, whereas no inactivation occurs at  $p_H$  7.3. Symptoms of poisoning can only occasionally be produced in guinea-pigs and rabbits when toxin is introduced directly into the stomach. Uniformly positive results are obtained in experiments in which the reaction of the stomach is adjusted to  $p_H$  7.3 at the time the toxin is introduced. The animals die within five days, and postmortem signs of acute gastro-enteritis and marked congestion of internal organs, associated with hemorrhage in the stomach and kidneys, are evident. Symptoms of poisoning can be produced by intrarectal inoculation of toxin when the rectum has been irrigated with saline solution and the reaction adjusted to  $p_H$  7.3. Postmortem features are similar to those obtained after introduction of toxin into the stomach. This method is not so effective as inoculation into the stomach.

AUTHOR'S SUMMARY.

THE ENZYMES OF THE AGALACTIA VIRUS. A. PIRIE and B. E. HOLMES, Brit. J. Exper. Path. **14**:290, 1933.

The virus of Agalactia, when centrifugated out of a culture, can be shown to reduce methylthionine chloride, U. S. P. (methylene blue) in the presence of lactate, broth or serum. Growth of the virus in culture can be demonstrated by the

increased rate of reduction of methylthionine chloride by a measured amount of culture. A culture of *Agalactia* shows a large uptake of oxygen, and so also does a saline suspension of the virus in the presence of lactate or of broth. The dehydrogenase and oxidative systems are extremely sensitive to mechanical agitation. A saline suspension of the virus loses all its reducing capacity after air or nitrogen has been bubbled through it, or air in the presence of cyanide. The number of viable organisms is also much reduced. Broth has a protective effect on the enzymes and the viability. It is suggested that these facts may be of importance in connection with the filtrability of some other viruses. The enzyme activity and the viability are greatly reduced by exposure to light in the presence of very small amounts of methylthionine chloride. No change can be shown in the distribution of nitrogen in a culture during growth, and no disappearance of dextrose can be detected. There is nearly always a small decrease in lipid phosphorus during growth.

AUTHORS' SUMMARY.

AGGLUTININS WITH TYPHOID-PARATYPHOID VACCINE. G. GIGLIOLI, *J. Hyg.* **33**:387, 1933.

The author's results confirm Gardner's views: Inoculation with typhoid-paratyphoid organisms gives rise to somatic agglutinins. This reaction is marked in the first few weeks after vaccination, but it is always limited to low and medium dilutions; the highest titer for a positive result in this series, with an "O" antigen, was 1:320. In actual enteric infection "H" agglutinins are higher than "O"; but "O" titers are higher in patients with enteritis than in vaccinated subjects. In four cases of typhoid and in twelve of paratyphoid C, "O" agglutinins were found after the first week in dilutions from 1:80 to 1:2,560, and appeared earlier in the disease than the "H" agglutinins. "O" agglutinins in vaccinated subjects do not destroy the qualitative receptor analysis in the serologic diagnosis; quantitatively the end-titer must be established. Diagnosis by serologic methods within three months after inoculation is uncertain. After this period the presence of "O" agglutinins in high dilutions is suggestive of active infection, and negative results of tests have little value unless confirmed.

EDNA DELVES.

MULTIPLE TOXINS PRODUCED BY SOME ORGANISMS OF THE CL. WELCHII GROUP. A. T. GLENNY and others, *J. Path. & Bact.* **37**:53, 1933.

Culture filtrates of "*Bacillus paludis*" and the lamb dysentery bacillus contain at least four toxins for each of which there is a specific antitoxin. The existence of these toxins has been demonstrated by means of titrations by different methods against a number of serums. This has revealed the deficiency, in certain serums, of one or more of the specific antibodies. The relative proportions of each antibody in a serum depend chiefly on the normal antitoxic content before immunization of the horse providing the serum. The intravenous method of testing mice does not measure any single toxin-antitoxin reaction.

AUTHORS' SUMMARY.

SPECIES IMMUNITY TO VIRULENT STREPTOCOCCI. H. B. DAY, *J. Path. & Bact.* **37**:169, 1933.

When their virulence is exalted, streptococci become resistant to phagocytosis and to extraction with acid. Their agglutination reactions with antisera are also modified. These properties of virulent streptococci appear to be due to their power of forming a special material which is antigenic. This special antigen is common to the species and is formed by streptococci of different types—both hemolytic and nonhemolytic. In broth cultures this antigen is formed only by highly virulent strains during the phase of active growth and is almost entirely attached to the cocci. In serum-broth cultures this antigen is also present in the culture fluid and persists longer. This species or "V" antigen resembles other antigens of pyogenic cocci in that it is: (a) resistant to acid, (b) destroyed on

heating in an alkaline medium, (c) destroyed by exposure to the action of bacterial enzymes and (d) readily adsorbed to protein on precipitation of the latter. Injection of V antigen excites active immunity to all virulent streptococci, hemolytic and nonhemolytic, while the serums of animals so treated confer passive immunity.

AUTHOR'S SUMMARY.

A SIMPLE METHOD FOR PREPARATION OF SPECIFIC SOLUBLE SUBSTANCE OF TYPE I PNEUMOCOCCUS. H. W. DUDLEY and W. SMITH, *J. Path. & Bact.* **37**:341, 1933.

The method depends on the precipitation of the specific polysaccharide from aqueous solution by 1.5 volumes of 96 per cent alcohol, repeated precipitation eliminating most of the accompanying contaminations. It is said to be a reliable method.

THE SEROLOGICAL GROUPING OF THE STARCH FERMENTING STRAINS OF *C. DIPHTHERIAE*. J. O. EWING, *J. Path. & Bact.* **37**:345, 1933.

One hundred and six starch-fermenting strains of *Corynebacterium diphtheriae* have been serologically tested and found to comprise five distinct types. The members of types A, B and D conform to the original description of the gravis organisms. Those of type C have an "atypical" colony form by which they may usually be distinguished. The fifth type (X) did not conform culturally to the description of the gravis organisms. The fifty strains which did not ferment starch were found to be serologically distinct from the starch-fermenting strains.

AUTHOR'S SUMMARY.

ANTIHAEMOLYTIC TITRE OF SERUM FOLLOWING STAPHYLOCOCCAL INFECTION. J. I. CONNOR and M. MCKIE, *J. Path. & Bact.* **37**:353, 1933.

Our results indicate that though there is much individual variation, superficial infection in man is accompanied in a high proportion of cases by a rise in the antihemolytic titer of the serum. The results of the treatment in these cases by injection of toxoid, recorded elsewhere, show that a rise in antihemolytic titer accompanies improvement or cure. We are not, however, prepared to assume that these parallel observations are necessarily correlated, and the improvement in clinical condition may be due to allergic desensitization or to some other cause. In three cases of pyemia in man a high antihemolytic titer was found—it was highest in one who survived the infection—but in the artificially immunized animals there was no clear relationship between the antihemolytic titer of the serums and indefinite or prolonged survival following the intravenous injection of living virulent staphylococci. It is, however, of interest that three animals indefinitely survived the intravenous injection of 0.5 cc. of a virulent broth culture of staphylococci.

AUTHORS' DISCUSSION.

SPECIFICITY OF BACTERICIDAL PROPERTIES OF NORMAL SERUM. M. H. FINKELSTEIN, *J. Path. & Bact.* **37**:359, 1933.

A nonspecific soluble factor is present in suspensions of heated bacterial cultures which may produce inhibition of the bactericidal action of normal serum. Nonspecific results sometimes obtained when normal guinea-pig serum is "adsorbed" with a bacterial culture and then tested for bactericidal properties are apparently due to this factor. Such effects may obscure the specific absorption of a natural bactericidal antibody by the bacterial antigen. Some samples of guinea-pig serum are unaffected by this factor at 0 C. but are susceptible to it at a higher temperature, e. g. 15 C. The inhibitory reaction produced by this factor is relatively slow as compared with specific absorption.

AUTHOR'S SUMMARY.



THE BACTERICIDAL POWER OF NORMAL SERUM. J. GORDON, *J. Path. & Bact.* 37:367, 1933.

Adsorption of both normal and heated serums by dead bacteria fails to yield any evidence for the existence of a series of specific antibodies in serum. The loss of bactericidal power consequent on adsorption is never specific for the adsorbing organism but is always general. The behavior of adsorbed heated serum is especially important. This is also true of pig's serum in spite of the fact that pig's serum contains some specific agglutinins. It was not found possible to demonstrate any sensitization of living bacteria by heated serum. The results recorded in this article lead to the belief that bactericidal activity is the result of the joint action of complement and a nonspecific heat-stable intermediary factor. It is believed that this factor is adsorbed by the bacteria, but that possibly, owing to its nonspecific character, the adsorption is only feeble and is not necessarily a primary adsorption. The experimental demonstration of such an adsorption would therefore be difficult.

AUTHOR'S SUMMARY.

TUBERCULOUS BACILLAEMIA. G. S. WILSON, Medical Research Council, Special Report Series, no. 182, London, His Majesty's Stationery Office, 1933.

Critical examination of available records leads to the conclusion that tuberculous bacillemia is far less frequent than has been claimed by many workers. It appears that bacillemia of an intensity sufficient to be detectable by present methods of examination is, except as a transitory phenomenon, rarely present in tuberculosis until the disease has assumed a more or less acute phase accompanied by extensive lesions or by actual generalization. It is present, perhaps, in from 5 to 10 per cent of cases of severe, advanced and progressive pulmonary disease and in from 30 to 40 per cent of cases of miliary and meningeal tuberculosis. In the cardiac blood of patients who have died of tuberculosis, postmortem examination may be expected to reveal the presence of tubercle bacilli in about 50 per cent of the cases. There is practically no evidence that a tuberculous bacillemia ever occurs in such diseases as articular rheumatism, polyarthritides, chorea, multiple sclerosis, schizophrenia, retrobulbar neuritis, Hodgkin's disease and certain diseases of the skin in the absence of gross lesions of tuberculosis, nor are there any sound reasons to suppose that the tubercle bacillus plays any essential rôle in the etiology of these diseases. The excessive numbers of positive results recorded by some workers are attributable to the failure to realize, or to pay adequate attention to, the numerous fallacies attending the demonstration of the tubercle bacillus. In this connection may be mentioned: (a) the occurrence in lysed blood of acid-fast particles closely resembling tubercle bacilli; (b) the failure to take the elementary precautions necessary to avoid confusion caused by the use of old slides, of blotting paper or of contaminated cedar-wood oil; (c) the impossibility, on microscopic grounds alone, of distinguishing the tubercle bacillus from saprophytic acid-fast bacilli that may gain access to preparations of suspected material from such situations as water, dust, excreta, sebaceous material and animal fodder, in which they are often abundant; (d) the assumption that macroscopic colonies of acid-fast bacilli appearing in culture are necessarily those of tubercle bacilli; (e) the diagnosis of tuberculosis in guinea-pigs following inoculation, on insufficient grounds, such as a microscopic demonstration of acid-fast bacilli in viscera of animals showing no macroscopic lesions or in the local lesion or regional lymphatic glands of animals showing no other evidence of the disease, or such as macroscopic lesions due either to spontaneous tuberculosis or to *Salmonella*, *Pasteurella*, *Brucella* or pyogenic infections, or such as a reaction to tuberculin in an animal that is found at autopsy to show no signs of tuberculous disease. In regard to the work of Löwenstein, it is impossible on the evidence presented to accept the far-reaching conclusions, both of fact and of theory, that he has put forward. There is, moreover, no reason to believe that isolation of the tubercle bacillus from the blood is likely to be of any practical value in the early diagnosis of tuberculosis.

FROM THE AUTHORS' SUMMARY.

PRECIPITIN REACTIONS IN THE BLOOD OF RHEUMATIC PATIENTS FOLLOWING ACUTE THROAT INFECTIONS. B. SCHLESINGER and A. G. SIGNY, *Quart. J. Med.* **2**:255, 1933.

Streptococcic precipitins can be demonstrated in the blood of rheumatic patients following acute streptococcic infections of the throat. Their formation is delayed until from the second to the fourth week from the onset of the nasopharyngeal infection (the end of the silent period), and their appearance in most cases foreshadows a tendency to a relapse of acute rheumatism. The precipitin corresponds to the type of streptococcus responsible for the infection, but a certain amount of formation of cross-precipitin may occur. The formation of precipitin is regarded as one of the manifold reactions which take place in the patient's defense mechanism during the silent period. Time is thus available for prophylactic measures if the infection of the throat has not passed unnoticed. Concentrated acetylsalicylic acid therapy during this period undoubtedly prevents serious relapses in many cases. It is not infallible, and further researches for a more reliable method are urgently required.

AUTHORS' CONCLUSIONS.

PARASPECIFIC IMMUNITY FROM BCG. A. CALMETTE and A. SAENZ, *Ann. Inst. Pasteur* **50**:433, 1933.

Experimental and clinical observations have demonstrated that four or five weeks following the injection or ingestion of BCG vaccine there exists an immunity to diseases other than tuberculosis, notably to streptococcic, pneumococcic, brucella and anthracotic infections. Calmette and Saenz believe that this accounts for the general reduction in mortality from all causes in vaccinated infants.

FROM THE AUTHORS' CONCLUSIONS.

ASYMPTOMATIC ANTHRACIC INFECTION IN MAN. G. J. SINAI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:199, 1933.

The population of a camp of Kossacks ate veal from a calf killed while it was sick with anthrax. Only one participant contracted the disease; he recovered in twelve days. Precipitation tests according to Ascoli were carried out on the other members of the tribe and on many inhabitants of the region. The blood serums of the tested persons were employed as antigen with a precipitating serum known to be specific. The result with the serums of fifteen of the thirty-eight tested persons was positive. Thirteen of the persons whose serums reacted positively ate the infected meat while of the 23 whose serums reacted negatively 17 ate the meat. The serums of a large number of the controls reacted negatively. Sinai concludes that consumption of meat infested with *Bacillus anthracis* may lead to an asymptomatic form of the disease. The positive reaction of the serums of a few persons was found to have become negative after one month's interval.

I. DAVIDSOHN.

INFLUENCING RESISTANCE TO *BACILLUS COLI* WITH OTHER BACTERIA. S. NUKADA and K. FUJII, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:287, 1933.

One hundred and twenty-two rabbits were treated with cultures of fifteen different bacterial species and on the tenth day after the last injection were given a fatal dose of a highly virulent culture of *Bacillus coli*. Their resistance was markedly increased by treatment with cultures of *Bacillus proteus*, *Bacillus pyocyaneus* and *Bacillus typhosus*, and to a lesser extent by treatment with cultures of *Bacillus paratyphosus* A and B, *Bacillus pertussis*, *Bacillus pestis* and *Meningococcus*; it was lowered by treatment with cultures of *pneumococcus*, *Staphylococcus*, *Bacillus influenzae* and *Bacillus dysenteriae*. The resistance of the rabbits was not altered by treatment with cultures of *Gonococcus*, *Streptococcus* and *Vibrio cholerae*. The influence was assumed to be of a nonspecific nature.

I. DAVIDSOHN.

SUPRARENAL GLAND AND INFECTION. H. LANGECKER and E. SINGER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **79**:326, 1933.

Suprarenal cortex and liver fed to mice for seven days increased their resistance to streptococcic and pneumococcic infections. Extracts of the organs acted similarly, but only if proper quantities were injected. Suprarenal cortex proved more potent than liver. The resistance of guinea-pigs to tuberculosis and typhus fever was not influenced.

I. DAVIDSOHN.

## Tumors

EXPERIMENTAL STUDIES ON LYMPHOMATOSIS OF MICE. J. FURTH, H. R. SEIBOLD and R. R. RATHBONE, *Am. J. Cancer* **19**:521, 1933.

Leukemic lymphomatosis (lymphoid leukemia), aleukemic lymphomatosis, lymphosarcoma and leukosarcoma are malignant processes; they are different manifestations of the same disease. Two factors are chiefly concerned in determining the type of the disease: (1) the possibility of free entrance of malignant lymphocytes into the circulation; (2) the character of the malignant lymphocyte. These factors alone are not quite sufficient to explain the individual variations of the blood involvement and of the localization of lesions in animals inoculated with the same strain. Lymphomatosis is transmissible to healthy animals, the success of transmission depending on the transfer of viable cells. Blood cells readily transmit lymphomatosis of mice, but cell-free plasma fails to transmit the disease. Procedures which destroy these cells but which fail to affect most viruses, such as adding glycerin or drying, inactivate the transmitting agent. Malignant lymphocytes are well preserved at low temperatures, and when kept in an icebox at  $+4^{\circ}\text{C}$ . for as long as seven days transmit the disease, but at temperatures below  $-20^{\circ}\text{C}$ . these cells are destroyed in thirty minutes. At incubator temperature, namely  $37.5^{\circ}\text{C}$ ., they lose their transmitting power in from three to twenty-four hours. Leukemic lymphocytes are not lymphoblasts but malignant (pathologic) lymphocytes with limited power of maturation, and when these cells multiply, their characteristics persist. These characters determine their ability to produce tumors, to invade the blood stream, to transmit the disease to related or unrelated mice, to localize in certain organs, etc. The essential change in leukemia consists not in the appearance of immature blood cells in the circulation (analogous to the "shift to the left" of Arneht) but in the invasion of the blood stream by malignant lymphocytes. The success of inoculations depends on the characters of the malignant lymphocytes persisting through successive passages, as well as on external factors such as the dose, the route of entry and the resistance of the mice. Furthermore, successful inoculation is dependent on an inherited susceptibility of the host. A single exposure to x-rays is sufficient to destroy the resistance of insusceptible mice to implantation of leukemic cells. The smallest quantity of irradiation that brings about this change is approximately 30 roentgens, and the percentage of successful inoculations increases with the quantity of irradiation. The duration of increased susceptibility depends on the quantity of x-rays used, and mice exposed to sublethal doses of x-rays may remain susceptible for three months. Irradiation of mice with sublethal doses immediately after inoculation does not destroy all the malignant cells that have been introduced and does not prevent fatal disease, but prolongs the duration of the illness by from 20 to 50 per cent, probably by partial destruction of the malignant cells.

FROM THE AUTHORS' CONCLUSIONS.

HEMANGIO-ENDOTHELIOMA OF THE LIVER. RALPH H. KUNSTADTER, *Am. J. Dis. Child.* **46**:803, 1933.

A white girl, weighing a little more than 8 pounds (4,428 Gm.) at birth, although showing persistent extreme pallor, seemed to develop normally until 6 weeks of age, when progressive enlargement of the abdomen began. Attacks of dyspnea and cyanosis occurred, anemia appeared, and later, constipation and recurrent vomiting. Exploratory operation at 5 months of age revealed a greatly

enlarged liver extending down on the right side to the iliac crest and on the left to the level of the umbilicus. The surface of the liver was covered with numerous purplish-red nodules varying in size from that of a pea to that of a walnut. They were soft and somewhat fluctuant. On aspiration a bloody fluid was obtained. Death occurred in twenty-four hours. The liver weighed 765 Gm. On section many of the nodules consisted of cystlike spaces filled with partially clotted blood. Microscopically, the hepatic tissue, which showed marked fatty changes, was compressed by groups of flattened or cuboid endothelial cells which lined networks of vascular spaces of varying sizes. In some areas the lining cells formed papillary ingrowths; in others, they had proliferated in compact masses. In many fields the vascular spaces were found within the hepatic parenchyma with no tendency toward formation of a capsule. Metastases were not found. Fourteen other cases of hemangio-endothelioma of the liver were found in reviewing the literature. Metastases occurred in 33 per cent of these cases.

RALPH FULLER.

THE CANCER CELLS OF SEROUS EFFUSIONS. G. S. GRAHAM, *Am. J. Path.* **9**:701, 1933.

Neoplastic cells are readily demonstrable in pleural and peritoneal transudates resulting from neoplastic disease of the serosae. They are of interest not only because of their immediate value in clinical diagnosis but also because of the opportunity they afford for cytologic study. The tumor cells are believed to proliferate more or less actively within the fluid in which they are suspended. They occur as single cells or as clumps showing a more or less marked tendency toward organoid arrangement. Tumor giant cells occur not infrequently and may be present in large numbers. While their formation probably depends on unfavorable conditions of growth, the phenomenon may be for the individual cells a purposeful and protective mechanism. These giant cells appear to result from successive fusions between individual cells, with the gradual building up of great nuclear masses of varying form. It is suggested that multipolar mitosis is a result rather than a cause of giant and multiple nucleation of tumor cells. Multipolar mitosis is followed in some cases by cellular disintegration. No figures definitely identifiable as phases of nuclear reconstruction after multipolar mitosis have been seen in the present preparations.

AUTHOR'S SUMMARY AND CONCLUSIONS.

CELL CHANGES IN THE ANTERIOR HYPOPHYSIS AFTER CANCER IMPLANTATION IN RATS. M. F. GUYER and P. E. CLAUS, *Anat. Rec.* **52**:225, 1932; **56**:373, 1933.

Bits of rat carcinoma transplanted to young rats of our stock developed into active cancer in from 80 to 90 per cent of these animals. Much the same structural and physiologic changes occurred in the anterior lobe in the cancerous rats as follow castration of either males or females, namely, increased number of basophilic cells, vacuolation of basophilic cells and increased potency of the transplanted substance in stimulating ovarian size and sexual precocity. A Golgi-like apparatus was prominent in both the basophilic and the oxyphilic cells. This apparatus was closely associated with the formation of the vacuolar material of the basophilic cells in both the cancerous and the castrate rats. A distortion of the nucleus—usually an indentation on the side turned toward the Golgi-like apparatus—occurred in the larger and presumably older basophils of both the castrates and the cancerous rats; this condition was much more prevalent in the cancerous rats. That some influence emanating from the cancerous growth operated directly on the pituitary anterior lobe instead of through a sterilizing effect on the gonad is indicated by the following facts: (a) The estrual cycle showed little or no irregularity and cancerous animals mated and bore young. (b) No follicular atresia was found in rats with even advanced cancer. (c) Transplanted substance from the anterior lobes of cancerous castrates was more potent in stimulating ovarian growth and sexual precocity in immature rats and mice than was that from the anterior lobes of mere castrates.

Following implantation of the Flexner-Jobling carcinoma in the uteri of rats there was a slight increase in the number of oxyphilic cells in the anterior lobes of their pituitary bodies. The histologic picture was the same as that associated with pregnancy or with injections of estrin. Following such implantation in castrated females there was not so great an increase in the number of oxyphilic cells. Daily injections of 4 rat units of a water-soluble preparation of estrin (theelin) did not prevent the formation of castration cells in the hypophysis of either the castrated animal or that with subcutaneous cancer, although such treatment kept the females in a state of almost continuous estrus. The estrual cycle continued in rats with transplanted uterine carcinoma and sections of the ovaries of such rats disclosed the usual histologic picture of the nonpregnant rat.

FROM THE AUTHORS' SUMMARIES.

**SPHENO-OCCIPITAL CHORDOMA.** MEYER CANTOR and LOUIS D. STERN, Arch. Neurol. & Psychiat. **30**:613, 1933.

Chordoma from the notochordal remnants may be an accidental finding; that is, it may give no symptoms, or it may give a variety of clinical pictures, depending on the location. In the case reported the clinical picture was made up of pain (aching) in the right half of the face, decrease of visual acuity in the right eye and signs of a slight involvement of the third nerve with those of increased intracranial pressure (absence of the posterior clinoid processes). A mass was seen pushing into the oral cavity from the right palatal region. It protruded externally in the region of the zygomatic process of the maxillary bone. There were loss of taste in the anterior part of the tongue, immobile palate, dysphagia and anesthesia of the right half of the face. Necropsy revealed a tumor protruding from the right palatal region into the oral cavity. It filled the right orbital and nasal cavities and the maxillary, ethmoid and sphenoid sinuses, destroyed most of the base of the skull and pressed on the right hemisphere, involving the second, third, fourth, fifth, sixth and seventh nerves. The tumor was vascular; calcium salts were deposited in the septums which divided the tumor into lobules. These contained physaliform cells, which were arranged in columns and were mostly vacuolated. Many of the vacuoles contained shrunken cells that resembled cartilage cells. There were no metastases.

G. B. HASSIN.

**ASTROCYTOMA OF THE CEREBELLUM: SURVIVAL FOR FORTY-FIVE YEARS WITHOUT OPERATION.** L. HAUSMAN and L. STEVENSON, Arch. Neurol. & Psychiat. **30**:1100, 1933.

The first symptoms of cerebellar tumor appeared between the ages of 6 and 8 years, but the diagnosis of tumor of the brain was not made until the age of 16. For twenty-five years following antisyphilitic treatment with mercury and iodides the patient was practically well, notwithstanding that neither the marital history nor the serologic studies revealed syphilis. Occasional relapses could be controlled by antisyphilitic treatment. For two years before death the cerebellar, mainly the mental, symptoms became uncontrollable. Necropsy revealed a fibrillary astrocytoma of the fourth ventricle with cystic formations, which extended into the right and partly into the left hemisphere of the cerebellum. Another cystic cavity took the place of the sylvian aqueduct, and the third ventricle was distended and both foramina of Monro were enlarged. The pathologic conditions found at necropsy are assumed to date back to childhood.

G. B. HASSIN.

**NASOPHARYNGEAL CARCINOMA.** O. CHRISTIANSON and S. W. McARTHUR, Arch. Surg. **27**:1109, 1933.

Three primary nasopharyngeal carcinomas with metastases to the cervical lymph nodes are described. The diagnosis for each patient was made clinically by examination of surgically excised tissues, and the body of one was examined post mortem. Histologically, all the tumors resembled immature squamous or pseudostratified epithelium, but in one the cells tended markedly to assume the arrangement and

structure of squamous epithelium. An essential clinical feature was the insidious and painless bilateral enlargement of the cervical lymph nodes due to metastases. The bilateral distribution of the metastases resulted from the midline location of the primary tumor and the dissemination of the growth into the lymphatics on both sides of the neck. Slight variations in cellular structure occurred in these carcinomas, but the variations were within the limits of the varieties of cells found in the pseudostratified epithelium lining the nasopharynx.

AUTHORS' SUMMARY.

COMPLEMENT FIXATION TEST IN CARCINOMA. W. SAPHIR and NELL HIRSCHBERG, *J. Immunol.* **25**:439, 1933.

The complement-fixation test has been studied in twenty-seven serums of cancerous patients and eighty-two control serums. The tests were positive in 77.7 per cent of the serums of cancerous patients. Only one of the eighty-two control serums, that from a patient with lobar pneumonia, gave a positive reaction. The authors are of the opinion that the reaction is physicochemical and due to the influence of the antigen on the globulins in the serum, rather than a specific antigen-antibody reaction. The clinical value of the reaction awaits further observation after refinement and after attainment of a better understanding of the nature and limitations of the test.

AUTHORS' SUMMARY.

SPECIFICITY OF IMMUNITY ELICITED BY MOUSE SARCOMA. H. B. ANDERVONT, *Pub. Health Rep.* **48**:1472, 1933.

Acquired immunity induced by propagable tumors is known to be effective against other transplantable growths. The purpose of the experiments recorded in this paper was to continue the earlier investigations pertaining to the specificity of the immunity elicited by sarcoma 180. The results attending the previous investigation and those recorded in this communication may be summarized as follows: 1. Sarcoma 180 induces resistance in mice which is effective against carcinoma A, carcinoma 63 and carcinoma 205. 2. It fails to induce any pronounced resistance to sarcoma 37. 3. Growth of carcinoma A, carcinoma 205 or sarcoma 37 in the mouse's tail elicits concomitant immunity in a considerable percentage of instances. However, the resistance induced by any of these tumors is not effective against sarcoma 180.

AUTHOR'S SUMMARY.

THE FUJINAMI MYXOSARCOMA IN DUCKS. W. J. PURDY, *Brit. J. Exper. Path.* **14**:297, 1933.

Normally, adult ducks recover from a Fujinami infection and are then solidly immune to the disease. It has now been shown that this immunity to subsequent infection is well developed by the third day, and that immunity becomes complete within the following few days. It has now been shown, too, that Rous sarcoma no. 1 growing in a duckling does not induce immunity to a Fujinami infection.

AUTHOR'S SUMMARY.

TUMORS OF THE TESTIS. A. PRUSZCZYŃSKI, *Virchows Arch. i. path. Anat.* **290**:137, 1933.

Pruszczyński describes four malignant tumors of the testis and presents a general consideration of testicular neoplasms based on the French and German literature. Tumors of the testis may be simple, mixed or teratoid. The simple tumors may be benign or malignant epithelial neoplasms or benign connective tissue tumors. The occurrence of sarcoma of the testis is doubtful. The malignant epithelial growths are divided into those with origin from the wolffian duct, of which one is described, and those originating in the seminal tubule epithelium. Tumors of the latter division he subdivides into seminomas of mesoblastic type, of spermatogonial type, of Sertoli cell type and of mixed type. The seminomas of mesoblastic type are composed of small, deeply stained cells derived from the undifferentiated mother cells of the tubules of the developing testis. Pruszczyński

describes one such tumor in the cryptorchid testis of a pseudohermaphrodite. The other two neoplasms which he describes in detail were spermatogonial seminomas. He appends descriptions of a benign and a malignant teratoma of the testis. He does not accept the view of French authors that such tumors arise from the primitive kidney.

O. T. SCHULTZ.

MYELIN SHEATHS IN GANGLIONEUROMAS. B. KARITZKY, *Virchows Arch. f. path. Anat.* **290**:161, 1933.

Three immature and four mature ganglioneuromas of a series of eighteen were especially examined for the presence of myelin sheaths. Sheaths were found in all the number varying with the degree of maturity of the tumor tissue. The sheaths were new formed. Their formation began in the more mature parts of the tumor and usually about blood vessels.

O. T. SCHULTZ.

CAVERNOUS HEMANGIOMA OF THE SUPRARENAL GLANDS. M. MÜLLER-STÜLER, *Virchows Arch. f. path. Anat.* **290**:177, 1933.

The condition described was an accidental finding at the necropsy of a woman aged 77 years. The suprarenal glands measured, respectively, 6.4 by 4.2 by 1.8 cm. and 6 by 3.5 by 1.3 cm. Each consisted predominatingly of cavernous blood spaces, in some of which thrombosis and calcification had occurred. The condition is held to have been due to congenital maldevelopment. No symptoms referable to the suprarenal glands had been noted during life.

O. T. SCHULTZ.

SEROLOGIC DIFFERENTIATION BETWEEN GLIOMATOUS AND NORMAL CEREBRAL PARENCHYMA. H. REICHNER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **80**:85, 1933.

In complement-fixation tests, the serum of one of three rabbits treated with gliomatous tissue reacted specifically with the homologous antigen (glioma), permitting a sharp differentiation of gliomatous from nondiseased cerebral tissue. Serum immunized against normal brain tissue reacted with normal as well as with neoplastic cerebral tissue. Some antisera produced with gliomatous tissue had the properties of organ-specific antisera. Attempts to make the antitumor serum absolutely specific by adsorption with brain tissue failed, because this removed all the antibodies.

I. DAVIDSOHN.

RELATION BETWEEN FOWL LEUKOSIS AND SARCOMA. A. R. MEYER and J. ENGELBRETH-HOLM, *Acta path. et microbiol. Scandinav.* **10**:380, 1933.

Erythroblastosis and myelosis of fowls are related to transmissible fowl sarcoma. The authors worked with a strain possessing both erythroblastic and sarcomatous properties. Intravenous injection of the material caused erythroblastosis; intramuscular administration induced sarcoma. Among seventy-two fowls with induced erythroblastic reactions there were fourteen with typical erythroblastic leukoses. There were sixty fowls with induced fibrosarcoma of characteristic destructive power. In twenty-one instances there were both sarcoma and erythroblastoma after intramuscular injection of the fresh tissue. In the viscera, endotheliomatous and angiosarcomatous changes were found. These changes are ascribed to action of the agent on immature mesenchymal cells (hemohistioblasts).

JACOB KLEIN.

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, Jan. 8, 1934*

E. H. HATTON, *President, in the Chair*

### OCCURRENCE AND SIGNIFICANCE OF GERMINAL CENTERS (FLEMMING) IN THE HUMAN SPLEEN. PAUL R. CANNON.

An examination of 416 human spleens was made with especial reference to the so-called germinal centers in the malpighian corpuscles. The object was to learn whether the secondary nodules are centers for the production of lymphocytes or centers of reaction to pathologic stimuli. The secondary nodules (more lightly stained areas composed of cells other than small lymphocytes) were arranged in three groups: (1) those consisting largely of fragmented lymphocytes, or polymorphonuclear leukocytes; (2) those composed predominantly of reticulum cells, and (3) those in which hyaline material was conspicuous.

Forty-four of the spleens contained such secondary nodules in considerable numbers. In the other 374 spleens, or 90 per cent, the malpighian corpuscles consisted largely of small lymphocytes. Of the abnormal secondary nodules, ten belonged to group 1, twenty-nine to group 2, and five to group 3. The secondary nodules occurred in patients varying in age from 5 months to 72 years, but about two-thirds of them in persons under 35 years of age. Practically all occurred in patients suffering from severe acute or chronic infectious or toxic diseases such as acute rheumatic fever, ulcerative endocarditis, generalized tuberculosis, generalized peritonitis, acute leptomeningitis, acute bilateral otitis media, glomerulonephritis or acute toxic necrosis of the liver. None or only occasional ones were found in patients who had died from carcinoma, either localized or generalized, lymphatic leukemia, Hodgkin's disease or hypertension.

Special stains, particularly Mallory's connective tissue stain and hematoxylin-eosin-azure, indicated that the "centers" in group 1 represented areas of acute localized inflammation, whereas those in groups 2 and 3 represented proliferative reactions with the formation of collagen or hyalin. Amyloid changes were also included in this group. Large and medium-sized lymphocytes were inconspicuous in all of the three groups.

These findings suggest that the tissue reactions described represent exudative and proliferative responses of lymphoid tissues to pathologic stimuli rather than centers for the active production of lymphocytes and add further evidence to the hypothesis of Hellman that they should be regarded more as "reaction centers" than as "germinal centers."

#### DISCUSSION

P. A. DELANEY: Did you have an opportunity to study developing germinal centers of the lymph nodes?

P. R. CANNON: No; only those in the spleen. Most studies have been made on lymph nodes.

P. A. DELANEY: As regards the question whether they were endothelial or lymphatic germinal centers, it is important to state that reticulocytes are not lymphoblastic.

### MONOCYTIC LEUKEMIA. VICTOR LEVINE.

In 1913 Reschad and Schilling reported a disorder which they designated "leukemia with transitional cells," a monocytic leukemia. Dameshek, in 1930, collected thirty-three instances, nineteen of which he considered authentic.



Considerable confusion exists about monocytic leukemia. First, there has been no agreement as to the origin of the monocytes. Some consider them derivatives from myeloblasts; others, from lymphocytes, and still others, from reticulo-endothelial cells. Secondly, monocytes and their derivatives, the circulating histiocytes, are often found in large numbers in the blood stream in infectious conditions, such as kala-azar, malaria and smallpox, many cases of which are erroneously considered as monocytic leukemia. Thirdly, true myelogenous leukemia may have a transient or permanent monocytic phase. Finally, many recent reports are too incomplete or indefinite for careful analysis.

In the last two years there have been five patients with monocytic leukemia and one with myelogenous leukemia with an acute monocytic phase at Grant Hospital and Cook County Hospital. These conditions have been verified post mortem. They occurred in more than 6,000 autopsies, fifty-six of which were in cases of leukemia. Of the fifty-six cases of leukemia, thirty-five were acute. In most of the acute cases the leukemia was myelogenous, disproving the statement of Pinkus, in 1905, that all acute leukemias are lymphatic.

In acute myelogenous leukemia there is an extensive proliferation of myeloblasts in the marrow, with a predominance of myeloblasts in the blood. In acute monocytic leukemia an analogous condition might be expected, such as a marked proliferation of the reticulo-endothelial cells in the marrow and a predominance of monoblasts (the immature precursors of monocytes) in the blood. This however does not occur. Marked proliferation of the reticulo-endothelial cells usually appears as a response to infectious or metabolic disorders and does not develop into leukemia; it is a reticulo-endotheliosis. Since it differs from leukemia, the term "reticulo-endothelial leukemia" should not be applied to monocytic leukemia.

In monocytic leukemia the blood differs from that in other leukemias in that the most numerous cells are the monocytes and their descendants, the circulating histiocytes. Monoblasts must be present for a diagnosis of leukemia, but rarely are they the most numerous of the cells. The monoblast is from 15 to 40 microns in diameter and has a narrow rim of deeply basophilic cytoplasm containing a round nucleus in which the chromatin has a spongy or sheepskin-like appearance.

Oxydase stains were used in studying the cases reported here. In all but one the monocytes in the blood stream contained oxydase granules, and in the microscopic sections of this one, many of the monocytes had oxydase granules. In one patient the oxydase reaction was marked; in most instances the monocytes had fewer oxydase granules than the cells of the myelocytic series. This discrepancy is unexplained at present. With the supravital stain in two cases many of the cells were typical monocytes and histiocytes.

Of the five cases of monocytic leukemia, two were in women. Four had short clinical courses, and one was known to have been present for four months. Complaints involving the nose and throat, such as epistaxis, bleeding gums and ulcerative gums, were frequent. Several patients had petechiae; in only one was the spleen easily palpable. All the patients had moderately severe anemia. The lowest white cell count was 4,400; the highest, 120,000. The differential count in most cases disclosed the monocytes as the most numerous cells, the percentage in one instance being as high as 70. The percentage of monoblasts varied from 7 to 37. Normoblasts were frequent in some instances, and a few immature granulocytes were present in several instances. In the case of myelogenous leukemia with a monocytic phase, while the monocytes were predominant in the stains of the blood there were also a few myeloblasts and myelocytes, as well as monoblasts.

At autopsy the marrow was hyperplastic; the weight of the spleen ranged from 50 to 1,000 Gm. Differential counts of the cells in the marrow were made in all except one case in which the fixation was poor. These counts are considered the final criterion in diagnosis. In the cases of monocytic leukemia the preponderant cells were the monocytes and immature monocytoïd cells. Their percentage varied from 52 to 77, whereas the highest percentage of cells of the granulopoietic series was 24. In the case of myelogenous leukemia with an acute monocytic phase, the cells of the monocytic series were 39 per cent while the granulopoietic cells were 42 per cent of the total.

## ARRESTED DEVELOPMENT OF THE PRIMARY EXCRETORY DUCT. C. W. APFELBACH.

The genito-urinary tract from a full-term male infant 1 hour old was demonstrated. The two soft and boggy kidneys were enlarged symmetrically to approximately five times the normal size. The markings of the fetal lobations were irregular. The hilus of each kidney was more in the anterior surface than in the medial. The two ureters were short and the lumens narrow. The urinary bladder was only 1.5 cm. long and the trigon, particularly, was minute. The urethra had a circumference of from 2 to 3 mm., and there was an epispadias. The testes had not descended into the scrotum. There was one renal artery for each kidney with a circumference of 3 mm. When the kidneys were sectioned in the usual way, the renal pelves were narrow tubes of the same caliber as the ureters. There were two short major calices also approximately the size of the ureters. There was an abundance of renal tissue. The cortex and the columns of Bertin were more distinct than the medullary portion. The cortex was made up of multiple minute cysts. In the medulla only a few large tubes were present.

The cortex contained many small glomeruli with dilated capsules and many tubules, all separated by an edematous stroma. In sections through the medulla there were only occasional tubules.

Inasmuch as the ureter, renal pelvis and collecting tubules, part of the trigon of the urinary bladder and the vas deferens are derived from the primary excretory duct, this anomaly is explained on the basis of arrested development of that structure.

## CALCIFICATION OF THE DURAL AND CEREBRAL TISSUE IN A HYDROCEPHALIC IDIOT CHILD. GEORGE RUKSTINAT.

A girl idiot, aged  $3\frac{1}{2}$  years, was the second child and the second idiot girl born to apparently normal parents. The first child developed poorly and died at the age of 3 years. Neither child had stigmas of mongolian idiocy. The second child died of bronchopneumonia and was markedly emaciated. Necropsy was performed about eight hours after death. The fontanels were closed, the cranial sutures were distinct, and the calvarium was symmetrical. The top half of the dura was opaque and glistening and in its dorsal third was about twice the normal thickness. The leptomeninges of the entire top of the brain were edematous; the convolutions were narrow and the sulci deep. The lateral ventricles were abnormally fluctuant from bilateral internal hydrocephalus, which had increased their maximum cross diameter to 7 cm. The larger arteries and veins of the brain were unchanged. On the right side, in the wall of the superior longitudinal sinus, 3 cm. anterior to the torcular Herophili, was a yellow-gray, firm mass 15 mm. long and from 1 to 2 mm. thick. Its long axis paralleled the sinus, and a small lateral projection lay in contact with one of the larger meningeal veins. Similar but smaller deposits occurred in the dorsal half of both cerebral hemispheres, especially at the junction of the gray and white matter. Two such masses formed ridges beneath the lining of the lateral ventricles. The masses were especially abundant in the dentate nuclei and could be traced in clusters about the main arteries in these structures. The deposits were hard and gritty with exception of the single mass in the dura.

Microscopically, the deposits contained calcium, which occurred as globules in the brain tissue and as cords along the capillaries and in the adventitia and media of the arteries. The globules formed conglomerate mulberry-like masses and, combined with short cords, formed structures like a child's jack with bulbous ends on thinner crossing and interlacing bands. In some arteries the lumen was compressed to half the diameter of a red blood cell. About the regions of densest deposit there was a marked capillary proliferation but only a slight increase in glia. Most zones about these deposits were, in fact, remarkable for their lack of reaction.

The other organs were altered only in a way compatible with the marked inanition and the bronchopneumonia. The thymus weighed only 8 Gm.

The presence of the calcified material in the outer portions of vessels and in the perivascular spaces was evidence, in some measure, of the route taken by the substance predisposing to calcification. The nature of this substance was not apparent but it seemed possible to postulate some injury to the brain at birth or as a result of the hydrocephalus which supplied the necessary base material in which calcium later accumulated. The limitation of these deposits to the brain and dura suggested a local chemical disturbance such as an altered carbon dioxide content of the tissues, although certain endocrine dysfunctions might have explained the condition.

#### DISCUSSION

R. H. JAFFÉ: The possibility of traumatic lesions may be considered. Lesions due to trauma of the brain at birth often cause different kinds of calcification.

G. RUKSTINAT: I have considered that. There were no reactions around the lesions.

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### PHILADELPHIA PATHOLOGICAL SOCIETY

*Regular Meeting, Jan. 11, 1934*

V. H. MOON, *President, in the Chair*

#### CYST IN A LARGE MYOMA UNDERGOING SARCOMATOUS CHANGE. LEWIS C. SCHEFFEY.

A Negress, married, aged 39, a secundipara, was admitted to the gynecologic service of the Jefferson Medical College Hospital, Jan. 9, 1933, complaining of menorrhagia, vaginal discharge, frequency of urination and abdominal enlargement. The menstrual disturbance and vaginal discharge had been present for three years; the irritability of the bladder and the abdominal enlargement were of one year's duration. Bimanual examination revealed a soft, fluctuating, freely movable and well-outlined enlargement occupying the entire pelvis and lower abdomen and extending above the umbilicus. Pregnancy and pelvic inflammatory disease having been excluded, a presumptive diagnosis of a soft myoma of the uterus or an ovarian cyst was made. The abdomen was opened on January 26, following a preliminary curettage of the uterus. The uterus was found uniformly enlarged, with a smooth, glistening surface; it had the size and appearance of a uterus seven months pregnant. The adnexae were normal. Supravaginal hysterectomy was done, the tumor being removed intact, with conservation of both adnexae.

On being incised on the anterior surface the tumor released 3,500 cc. of clear serous fluid similar to amniotic fluid. The wall of the uterus was markedly thickened, being made up of trabeculated and convoluted formations of fibrous tissue. This large cavity (12 by 8 by 8 cm.) in the uterine wall encroached on an elongated, thinned-out endometrial surface, but was encapsulated in the anterior uterine wall, being a so-called pseudocyst of the uterus; it was in a myoma that had undergone hyaline and cystic degeneration with liquefaction.

The histologic report (Dr. Crawford and Dr. Hoffman) was as follows: The uterine glands were beginning to assume serpentine formations and a few of the glands presented early secretory activity. Here and there a gland was cystically dilated and hyperplastic. The stroma was edematous and rather well vascularized. The myometrium was well vascularized and somewhat edematous. The fibrocyst was lined by a rather thick zone of cellular tissue, which was mostly made up of muscle cells intermingled with other types of cells such as spindle cells and round cells. This great cellularity gave the growth a malignant appearance. The variation in the chromatin content, size and shape of the nuclei and the mitotic figures fit into the picture of sarcoma. The clumping of cells was rather characteristic of sarcoma. This growth was more or less differentiated

in some areas from the underlying normal musculature. In some areas deep penetration by the sarcomatous growth was seen.

Postoperative roentgen examination revealed no metastatic areas in the lungs or bones. The patient was heard from recently and had remained well. This case is reported in *Surgical Clinics of North America* (14:143 [Feb.] 1934).

The occurrence of a large cystic myoma has been reported by R. T. Frank. He mentioned enormous tumors reported by Lihotsky, containing 3.4 liters (3,400 cc.); Lingens, weighing 45 pounds (20.4 Kg.); Webster, 87 pounds (39.4 Kg.), and Kelly and Cullen, 89 pounds (40.4 Kg.). In none of the reports of these cases was the occurrence of sarcomatous degeneration mentioned as a complicating lesion. The resemblance to pregnancy may be striking. Hartman erroneously reclosed the abdomen in such a case (reported by Frank).

#### CARCINOMA OF THE TAIL OF THE PANCREAS ASSOCIATED WITH POLYPOSIS AND CARCINOMA OF THE STOMACH. JOHN W. PARSONS.

A Negro, aged 39, had had syphilis of the central nervous system for five years and epigastric pain with occasional attacks of vomiting for three weeks. He was admitted to the hospital of the University of Pennsylvania in September, 1933. He was poorly nourished, with signs of tabes dorsalis and optic atrophy, and had a diffuse mass in the epigastrium. His course was steadily downward and he died three months after admission to the hospital.

Autopsy revealed a large, firm, whitish-pink mass occupying the tail of the pancreas which was poorly defined from the surrounding tissue. The lymph nodes in the region of the pancreas were greatly enlarged owing to infiltration by similar firm tissue. The liver was studded with nodules of tumor tissue, and these were confluent over a large area in the right lobe. The peritoneal surfaces of the intestine contained many small nodules, and similar tissue was observed in the suprarenal glands and kidneys.

The stomach was greatly dilated and its wall thickened. The mucosa was thickly studded with small, moderately firm, polypoid masses varying from a few millimeters to 1 cm. in diameter. There were three large ulcers on the mucosa, two of which were covered by unbroken epithelium and were apparently healing. The third was larger than the others; its center was covered by a thin layer of dirty brown necrotic material, and its edges were slightly elevated above the adjacent mucosa. The pylorus was the site of a ring of large confluent polyps which almost completely occluded its lumen.

The mass in the pancreas was composed of large, poorly differentiated, actively growing epithelial cells. There was some tendency toward glandular structure, though many areas were solid. The stroma was moderately dense with few large blood vessels. The nodules in the lymph nodes, liver, intestine, suprarenal glands and kidney conformed to this histologic structure.

The mucosa of the stomach was greatly hyperplastic, with considerable increase in the size of the epithelial cells, which contained numerous large vacuoles. The polyps were composed of such epithelium and were supported on a narrow stalk of loose fibrous tissue. Sections from the ulcerated area showed the center to be denuded of epithelium and covered by necrotic debris. The edges revealed irregular hyperplasia of the epithelium, which penetrated the basement membrane in places and extended in irregular cords into the submucosa. Under the center of the ulcer such epithelial overgrowth was especially conspicuous, and here there also was penetration of the muscularis mucosae.

There were several areas in the stomach in which cords of epithelial cells were found growing within lymphatic vessels in the subserosa. These cells resembled the cells of the tumor of the pancreas more than they did those of the carcinoma of the stomach.

The mass in the pancreas was a primary carcinoma of poorly differentiated type. The ulcerated area in the stomach showed unmistakable signs of carcinoma primary at that site. The polyps on the gastric mucosa showed no tendency toward

malignancy. The metastatic tumor nodules all resembled the pancreatic carcinoma rather than that of the stomach. It is of special note that metastases from the pancreatic tumor were found in the stomach, itself the site of a carcinoma.

ACUTE AMEBIC DYSENTERY. F. L. HARTMAN and CLARKE E. BROWN.

A white man, 41 years of age, was admitted to the Lankenau Hospital Aug. 27, 1933. He had attended a convention in Chicago from June 21 to June 26. The date of onset of the symptoms was not recalled, but during July he experienced severe attacks of abdominal pain, without nausea or loss of appetite. After the first of August he noted increasing fatigue but nevertheless continued at work until August 20. The onset then was sudden with severe pain in the abdomen and intense constant desire to defecate. The bowel movements were about twenty in twenty-four hours, containing much mucus and little blood. There was practically no rise in temperature. Tenesmus was intense. There was no vomiting. The abdominal wall was fairly rigid and sensitive to the touch. These records were supplied by Dr. R. H. Bloss, of Bethlehem.

On admission the patient was moribund. According to his wife he had always enjoyed good health except for occasional attacks of epigastric distress coming on immediately after meals for the past four or five years. These were extremely mild and were always relieved by his taking a small amount of soda. He had never had diarrhea.

The patient was semistuporous; the skin was cold, clammy and pale. The heart was moderately enlarged; there were no murmurs but the muscle tone was decidedly poor. The abdomen showed moderate distention, generalized mild tenderness and slight rigidity. Peristalsis was increased.

On rectal examination pronounced boggiess of the wall was found just inside the sphincter, and as the finger was inserted farther it met with apparent obstruction. This obstruction was due to firm ridges with apparent excavations such as one finds in an ulcerative carcinoma. However, the feel was not nodular and not hard. It gave one the impression of intussusception with the edematous bowel extending down. There was a constant discharge from the rectum of a thin, brownish-black, watery, mucoid fluid, and although the patient had marked tenesmus and bearing down there were no large stools. Examination of the fluid from the rectum showed mucus, a heavy trace of blood, absence of bile, large amounts of pus, epithelial cells and granular debris. The reaction was alkaline. Parasites were not found. The patient's death occurred the next morning.

Autopsy (by Dr. Brown) revealed generalized purulent and pseudomembranous colitis with transverse but nonperforating ulcers destroying almost the entire mucosa. The lower 6 cm. of the ileum was also involved. In sections stained with hematoxylin-eosin, iron-hematoxylin, the Giemsa stain and thionine, *Endamoeba histolytica* was found in abundance in all three layers of the thickened intestinal wall. Other organs were not involved.

EFFECT OF RETICULO-ENDOTHELIAL CELL BLOCKADE ON THE FORMATION OF ANTIBODIES IN RABBITS. LOUIS TUFT, with the assistance of MARY MULROONEY.

Considerable difference of opinion exists concerning the relation of the reticulo-endothelial system to the formation of antibodies as determined by blockade of the cells with inert particulate material. In the experiments reported as yielding no evidence of such a relationship the fault in the opinion of Cannon and his co-workers was either in a failure to give sufficiently large amounts of the blocking material and to continue its administration throughout the experiment to insure continued saturation of newly differentiated cells or in the use of too few animals or too large a dose of antigen. Employing these principles, they were able to show definite inhibition of the formation of hemolysin after blocking the reticulo-endothelial system with india ink.

By employing the same type of technic with slight modification we determined the effect of blockade of the reticulo-endothelial cells with india ink and trypan blue on the formation of agglutinins to the typhoid bacillus and to the paratyphoid bacilli A and B and on the formation of complement-fixing antibodies to the typhoid bacillus, the production of such antibodies in forty rabbits being stimulated by intradermal and intravenous injections of from 1 to 3 doses of mixed typhoid vaccine. Examination of the tissues of the "blocked" rabbits revealed marked infiltration of the reticulo-endothelial cells with india ink.

The results of these experiments showed fairly conclusively that this type of blockade is effective in preventing the formation of agglutinins and complement-fixing antibodies against typhoid, paratyphoid A and paratyphoid B bacilli, as shown by a suppression of both the natural and the acquired agglutinins and likewise by a failure of control animals that had previously shown ability to produce antibodies to produce them after being "blockaded." This suppression was independent of the type of blockading material employed or the route of administration of the antigen, the agglutinins being suppressed with considerable ease.

The results of these experiments supply further evidence of the importance of the reticulo-endothelial cells in relation to the formation of antibodies and emphasize the point brought out by Cannon and his co-workers that in all blockading experiments, irrespective of the material or the antigen employed, continued saturation of the reticulo-endothelial cells to suppress the activity of newly regenerated cells is essential for satisfactory results.

IMMUNIZATION OF MONKEYS AGAINST ACUTE ANTERIOR POLIOMYELITIS WITH  
SPECIAL REFERENCE TO ORAL IMMUNIZATION. JOHN A. KOLMER and  
ANNA M. RULE.

A chloroform-treated vaccine of monkey poliomyelitic spinal cord in a total dosage of 1 cc. by subcutaneous and intracutaneous injection failed to immunize two monkeys against intracerebral inoculations of the virus. Subcutaneous injections of a sodium ricinoleated vaccine appeared to produce slight immunity in one monkey while intracutaneous injections immunized two additional animals in a more convincing manner. Intracutaneous injections of an untreated vaccine successfully immunized one animal; when it was administered by a stomach tube it failed to protect two animals. A heated vaccine failed to immunize five monkeys when administered subcutaneously, intracutaneously and by stomach tube. Hexamethylamine, metaphen, mercurochrome, Pregl's solution of iodine (the solution resulting from the action of iodine on sodium carbonate solution), sodium and gold thiosulphate, sodium ricinoleate, neoarsphenamine, neutral acriflavine, tryparamide and bismuth potassium tartrate were ineffective in the treatment of experimental poliomyelitis in monkeys. From five to eight intravenous injections of each compound were given at intervals of three days in doses per kilogram of body weight comparable in most instances to the maximum amounts given adult human beings in the treatment of various diseases.

## Book Reviews

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**Diet and the Teeth: An Experimental Study. Part III. The Effect of Diet on Dental Structure and Disease in Man.** By May Mellanby. Medical Research Council Special Report Series, No. 191. Price, 5 shillings. Pp. 180, with 46 plates. London: His Majesty's Stationery Office, 1934.

The author's former work led naturally to the study of the teeth of man. In this comprehensive report, replete with illustrations, the author points out that the procedure in man necessarily must be different from that in experiments on animals. She first describes the structure of a perfect tooth. She then reports the results of her investigations on hypoplastic teeth. It was found that a close correlation exists between external appearance and internal structure, and that there is a definite relationship between caries and hypoplastic teeth. For example, in 1,500 deciduous teeth which were subjected to a comprehensive examination, 78 per cent of those with normal dentin were free from caries, while only 6 per cent of very hypoplastic teeth were free from caries. Although the evidence for a relationship between hypoplasia and caries is weighty, the condition of many teeth could not be explained by this hypothesis. Further investigation revealed that nearly all the exceptions to the first hypothesis could be explained by assuming a change in the character of the tooth subsequent to eruption. It was thought that this change in the nature or resistance of the tooth could be accurately determined by the presence and character of the secondary dentin. Thus, the author found that only 32 of 1,500 deciduous teeth were exceptions to the two general hypotheses, namely, that primary structure and caries are directly associated, and that the reaction of teeth to caries or attrition is modified after eruption, as is indicated by the presence and character of the secondary dentin.

Next she reports dietary experiments on groups of children in order to determine whether the incidence and progress of caries are affected by diets which, in general, are rich in vitamin D, calcium and phosphorus and low in anticalcifying substances. Although the number of children in these experiments was not great, and the duration was somewhat limited, she considers the results sufficiently striking to indicate that her previous experiments on animals are reliable guides to the study of dental disease in man.

The work covers a wide field, as the author also investigated the relationship between caries and rickets and found that a group of rachitic children had an extremely high incidence of caries. As to the geographic distribution of caries, it is pointed out that there is a strong tendency for caries to develop in natives who change their way of living to that of civilization in an amazingly greater frequency than when they live in their primitive state. The importance of vitamin D, calcium and phosphorus, together with common and prolonged breast feeding, as factors tending to produce a low incidence of caries was shown in this geographic survey, as was the tendency for diets high in anticalcifying substances, as, for instance, cereals, to produce a higher incidence of caries.

This important contribution is encouraging. However, some parts of the work seem to be on somewhat firmer ground than others. It seems logical to accept the fact that a tooth would be defective if the cells were not supplied with proper materials when it was under construction, and that such a tooth would be more susceptible to caries than a normal tooth. It also seems probable that the mineral content of teeth is dependent on the mineral metabolism, and so the use of proper diets can definitely influence the incidence and spread of caries.

The portion of the work concerned with the two hypotheses is somewhat confusing, because each hypothesis tends to weaken the other. A group of teeth cannot be divided so that the condition of those of one group may be explained by one hypothesis and those of the other by a second. Both primary structure and modification of structure after eruption must be considered as factors in every

tooth, which leaves one wondering which is the more important, primary structure or subsequent diet. The statistics of the use of secondary dentin as a useful guide in determining the resistance of hypoplastic teeth are not particularly convincing. There are so many factors in the production of secondary dentin that its use as an index must always be difficult. The factors which lead to the production of the matrix are not the same as those which lead to good calcification. Indeed, in bone the reverse is found: an overgrowth of matrix is characteristic of rickets. Nevertheless, this is an important piece of work and one in which a great deal of evidence is presented of the relationship between diet, structure of the teeth and caries.

**Die pathologisch-histologischen Untersuchungsmethoden.** By Prof. Dr. G. Schmorl, Geh. Medizinalrat und Direktor der patholog-anatom. Abteilung am Stadtkrankenhause Dresden-Friedrichstadt. Sechzehnte neu bearbeitete Auflage. Herausgegeben von Prof. Dr. P. Geipel. Price, 30 marks. Pp. 469. Berlin: F. C. W. Vogel, 1934.

Schmorl's textbook, for many years a reliable guide for methods used in pathologic laboratories, was revised for this edition by the author before his death in 1932; Geipel made a few editorial changes subsequently. The general arrangement of the material in eighteen chapters is comparable with that in the familiar American textbook by Mallory and Wright. The methods recommended are, however, described at greater length; many are modern. The eighteen chapters describe: the examination of unfixed preparations; fixation and hardening of tissues; decalcification and defatting; the method of making injections into blood vessels; sectioning of tissues; methods of preparing frozen tissue; embedding; the ordinary stains for tissue; impregnation with metals; clearing and preservation; the special stains for particular constituents of cells and tissues; the steps required for the examination of particular pathologic changes, individual tissues, such as nerve tissues, and organs and the blood, and the demonstration and identification of bacteria, the higher species of fungi and spirochetes. A final chapter describes how animal parasites are sought and how their characteristics are ascertained. This manual has become well entrenched as a valuable guide in pathologic histology by many previous editions. This edition equals the others in merit and usefulness.



## Books Received

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THE CHEMISTRY OF ANTIGENS AND ANTIBODIES. J. R. Marrack, D.S.O., M.C., M.D. Medical Research Council, Special Report Series, No. 194. Paper. Price, 2s. 6d. Pp. 135, with 26 illustrations. London: His Majesty's Stationery Office, 1934.

This little monograph represents the best attempt this reviewer has seen to apply modern physicochemical principles to an analysis of the fundamental nature of immune reactions. The first chapter, on physicochemical considerations, reviews modern theories of valence and molecular structure and discusses some of the properties of proteins that are fundamental to any critical examination of immune reactions. In chapter 2, on the nature of antibodies, the author discusses such matters as the separation of antibodies, the stability of antibodies and the composition of antigen-antibody complexes. Chapter 3 deals with the specificity of antigens and includes discussions of artificial protein antigens, polysaccharides and lipids, while chapters 4 and 5 are concerned with the nature of antigen-antibody reactions. Various types of immune reactions are discussed in the light of modern concepts of valence, molecular structure and molecular orientation as applied to the study of the structure and behavior of proteins. This is an extremely interesting volume and should be of especial value in directing the attention of students of immunology to the intricate physicochemical reactions which are behind the gross changes ordinarily observed in antigen-antibody reactions. There are extensive references to the original literature for those interested in pursuing this fascinating field.

REPORT OF THE MEDICAL RESEARCH COUNCIL FOR THE YEAR 1932-1933. Price, 2s. 6d. Pp. 161. London: His Majesty's Stationery Office, 1934.

ADDENDA TO A BIBLIOGRAPHY OF THE HONOURABLE ROBERT BOYLE. J. F. Fulton, M.D., Sterling Professor of Physiology, Yale University. Reprinted from the Oxford Bibliographical Society Proceedings and Papers, Volume III, part 3, pp. 339-365. Paper. Gratis. Pp. 27. New Haven: Yale University School of Medicine, 1933.

STUDIES ON HISTOMONIASIS, OR "BLACKHEAD" INFECTION, IN THE CHICKEN AND TURKEY. Ernest Edward Tyzzer, Department of Comparative Pathology, George Fabyan Foundation, Medical School, Harvard University. Price, \$1.25. Pp. 79, with 6 plates and 47 figures. Boston: American Academy of Arts and Sciences, 1934.

HYPOCHLORÉMIE ET ACCIDENTS POST-OPÉRATOIRES. ÉTUDE CLINIQUE PATHOGÉNIQUE ET THÉRAPEUTIQUE (COLLECTION MÉDECINE ET CHIRURGIE PRATIQUES, NO. 63). Mm. H. Chabanier et C. Lobo-Onell. Price, 22 francs. Pp. 146, with 9 figures. Paris: Masson et Cie, 1934.

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